

**The Biotic Ligand Model: Technical Support Document for Its
Application to the Evaluation of Water Quality Criteria for Copper**

U.S. Environmental Protection Agency
Office of Science and Technology
Health and Ecological Criteria Division
Washington, D.C. 20460

TABLE OF CONTENTS

1.0 INTRODUCTION	1
2.0 THEORETICAL BACKGROUND	2
2.1 Description of Generalized BLM Model Framework	2
2.2 Model Formulation	3
2.2.1 Chemical Model- Inorganic Metal Speciation	5
2.2.2 Chemical Model- Dissolved Organic Matter Complexation	5
2.3 Relationship of Copper Accumulation to Acute Toxicity	7
2.4 Description of Species on the Biotic Ligand	8
2.5 Alternative Computational Modes	8
2.5.1 Metal Speciation Computations	9
2.5.2 Metal Toxicity Computations	9
3.0 MODEL CALIBRATION	10
3.1 Sources of Thermodynamic Information	10
3.1.1 Inorganic Metal Speciation	10
3.1.2 Interactions of Copper with Organic Matter	10
3.2 Calibration of the BLM to Copper Accumulation at the Biotic Ligand	11
3.3 Response of the BLM to Variation in Hardness, DOC and pH	12
3.3.1 Effect of Dissolved Organic Carbon on Copper Accumulation and Toxicity	13
3.3.2 Effect of Hardness on Copper Accumulation and Toxicity	13
3.3.3 Effect of pH on Copper Accumulation and Toxicity	14
3.3.4 Summary	15
3.4 Application of the BLM to Fish Toxicity Data	16
3.4.1 Relationship of Biotic Ligand Accumulation Level to Effect	16
3.4.2 Evaluation of the Predicted LC50 Using the BLM	16
3.4.3 Application of the BLM to a Bioassay Dataset for Fathead Minnow With Water Treatments in Synthetic and Natural Waters	17
3.5 Parameter Estimation for Other Organisms	19
4.0 MODEL TESTING: A DESCRIPTION OF MODEL VALIDATION EFFORTS ...	20
4.1 WER Datasets	20
4.1.1 Model Testing - Water Effect Ratio Studies Using Fathead Minnow	20
5.0 MODEL APPLICATION	22
5.1 Applicability and Limitations	22
5.1.1 Equilibrium Assumptions	22
5.1.2 Aquatic Organisms	23
5.1.3 Ranges of Input Parameters Used in Calibration	23
5.1.4 Other Considerations	23

6.0 REFERENCES	24
-----------------------------	-----------

TABLES

- Table 1. Inorganic Copper and Organic Speciation Reactions in the WHAM Database (Tipping, 1994).
- Table 2. Stoichiometry and Thermodynamic Constants for Adsorption of Metals and Protons on Gills of Larval Fathead Minnows.
- Table 3. Average Composition of Waters Used in the Fathead Minnow Copper Toxicity Experiments of Erickson et al., (1987) in Exposures Without Chemical Modifications.

FIGURES

- Figure 1. Schematic diagram of the generalized biotic ligand model (BLM) framework for acute metal toxicity.
- Figure 2. Relationship between mortality of juvenile rainbow trout after 120 hours of exposure and copper concentration on the gill of the fish after 24 hours of exposure. Data from MacRae (1999).
- Figure 3. Calibration of the WHAM model, Version V. (A) Cu: effect of ionic strength: 0.001 M (○) 0.01 M (●) (B) Cu: effect of Ca: 0.001 M NaNO (○) 0.001 M Ca(NO) (●) 0.01 M Ca(NO) (□) (C) Cu: effect of pH: 5.14 (○) 7.00 (●) 8.44 (□) (D) Ca: effect of pH: 5.00 (○) 7.00 (●) 9.00 (□) (E) Cd: effect of pH: 4.00 (○) 6.00 (●) 8.00 (□) (F) Pb: effect of pH: 4.00 (○) 5.00 (●) 6.00 (□). Redrawn from Tipping and Hurley (1992).
- Figure 4. Schematic diagram of the biotic ligand model (BLM) framework for acute copper toxicity, showing inorganic and organic complexation in the water and interaction of metals and cations on the biotic ligand.
- Figure 5. Measured copper accumulation on fathead minnow gills from Playle et al., (12) and Biotic Ligand Model predictions as a function of cupric ion concentration.
- Figure 6. Relationship of copper LC50s to variations in DOC concentration. The lines are drawn by eye to represent the data. (A) LC50 expressed as the concentration of total dissolved copper. (B) LC50 expressed as the concentration of the free ion activity of copper. (C) LC50 expressed as the concentration of the copper sorbed to the gill. Data from Erickson (1996).
- Figure 7. Relationship of copper LC50s to variations in calcium concentration. The lines are drawn by eye to represent the data. (A) LC50 expressed as the concentration of total dissolved copper. (B) LC50 expressed as the concentration of the free ion activity of copper. (C) LC50 expressed as the concentration of the copper sorbed to the gill. Data from Erickson (1996).

FIGURES (continued)

- Figure 8. Relationship of copper LC50s to variations in pH. The lines are drawn by eye to represent the data. (A) LC50 expressed as the concentration of total dissolved copper. (B) LC50 expressed as the concentration of the free ion activity of copper. (C) LC50 expressed as the concentration of the copper sorbed to the gill. The LC50s in (B) are the measured copper activities using a specific ion electrode. Data from Erickson (1996).
- Figure 9. Response of measured and predicted fathead minnow Cu LC50 to changes in pH. BLM predictions assume that only Cu^{2+} is bioavailable.
- Figure 10. Distribution of complexed Cu^{2+} and CuOH^+ on natural organic matter as simulated in the WHAM.
- Figure 11. Distribution of adsorbed Cu^{2+} and CuOH^+ on gill membrane as simulated in the revised BLM.
- Figure 12. Response of measured and predicted fathead minnow Cu LC50 to changes in pH. BLM predictions assuming only Cu^{2+} is bioavailable are compared with the revised model that assumes both Cu^{2+} and CuOH^+ are bioavailable.
- Figure 13. Method of calculating the LC50 using the BLM. Relationship of copper concentration sorbed on the gill and dissolved copper as computed using the BLM for laboratory water and 100% effluent.
- Figure 14. Biotic Ligand Model predicted versus measured LC50 values for fathead minnow in static toxicity exposures from Erickson et al., (1996). Note that 1:1 (solid), 2:1 and 1:2 (dotted) reference lines are drawn for comparison.
- Figure 15. Simulated changes in free copper over time during a static toxicity test. Kinetics of copper binding to natural organic matter are based on results of Ma et al., 1999.
- Figure 16. Simulated changes in free copper over time during a static toxicity test with a 24 hour pre-test equilibration period. Kinetics of copper binding to natural organic matter are based on results of Ma et al., 1999.
- Figure 17. Simulated changes in free copper over time during a static toxicity test with a 24 hour renewal frequency. Kinetics of copper binding to natural organic matter are based on results of Ma et al., 1999.
- Figure 18. Simulated changes in free copper over time during a flowthrough toxicity test. Kinetics of copper binding to natural organic matter are based on results of Ma et al., 1999.
- Figure 19. Biotic Ligand Model predicted versus measured LC50 values for fathead minnow in flow-through toxicity exposures from Erickson et al., (1996). Note that 1:1 (solid), 2:1 and 1:2 (dotted) reference lines are drawn for comparison.

FIGURES (continued)

- Figure 20. Predicted versus measured values for *D. pulex* copper LC50 in Connecticut streams (23). Note that 1:1 (solid), 2:1 and 1:2 (dotted) reference lines are drawn for comparison.
- Figure 21. Comparison of BLM predictions for copper toxicity to a number of invertebrate aquatic organisms from the US EPA ambient water quality criteria document.
- Figure 22. The aqueous concentrations of TOC (taken to be equivalent to DOC in the application of BLM), alkalinity, hardness, and pH, in laboratory water (LAB), upstream water (U/S), and at the indicated percentages of effluent dilutions in upstream water. Data from Diamond et al. (1997).
- Figure 23. Comparison of measured and calculated LC50s and the water-effect ratios. Data from Diamond et al. (1997).
- Figure 24. Predicted versus measured values for fathead minnow copper LC50s in Water Effect Ratio studies (Diamond et al., 1997; Dunbar, 1996). The results from static exposures from Erickson et al., 1987 are included for comparison. Note that 1:1 (solid), 2:1 and 1:2 (dotted) reference lines are drawn for comparison.
- Figure 25. Predicted versus measured values for *C. dubia* copper LC50s in Water Effect Ratio studies (Hall et al. 1998). Note that 1:1 (solid), 2:1 and 1:2 (dotted) reference lines are drawn for comparison.

APPENDICES

- Appendix A. Chemical Speciation Equations for Copper

1.0 INTRODUCTION

The importance of explicitly considering bioavailability in the development of water and sediment quality criteria for metals has been recognized for some time (Di Toro et al., 1991 a,b; Allen and Hansen, 1996; Ankley et al., 1996). Criteria that incorporate this concept are being recommended to, and are being considered, for application by regulatory authorities (Bergman and Dorward-King, 1997; Renner, 1997). A long history of experiments demonstrates the importance of water chemistry on the degree of toxicity of metals. What has been missing is a practical modeling implementation that can predict these variations in toxicity with some degree of generality and reliability.

The Biotic Ligand Model (BLM) was developed in response to this need. The BLM represents a synthesis of ideas that have a long history of development. These ideas have been combined into an operational model of metal bioavailability and toxicity. The model is suitable for use in evaluating differences in the availability and toxicity of metals such as copper and other metals, differences that occur as a result of changes in water chemistry from site to site, and at a given site over time.

The BLM for copper have undergone an initial review by the USEPA Ecological Processes and Effects Committee Science Advisory Board (SAB; USEPA, 1999). The purpose of this SAB review was to assess the technical validity of the BLM approach. With regard to the question of whether or not the BLM could improve the Agency's ability to predict toxicity to water column organisms in comparison to the currently applied dissolved metal concentration criterion, the SAB concluded that the BLM could predict with "reasonable accuracy (generally within a factor of two of measured values the acute toxicities of copper and silver to fish" (USEPA, 2000)). The SAB also agreed that it had been shown to predict *acute toxicity to a limited number of water column organisms, for selected metals, under equilibrium conditions*. [italicized text reflects emphasis by SAB]. Further, it was concluded that the scientific underpinnings of the BLM appeared to be sound. It would not necessarily reduce uncertainty associated with metals bioavailability and toxicity as a site-specific adjustment, relative to empirical data, but "its predictiveness over a wide range of environmental conditions makes the BLM a more versatile and effective tool for deriving site-specific WQC." Finally, the SAB concluded:

"It appears premature to use the BLM to revise the protocol for deriving national ambient water quality criteria at this time, primarily because the model has not yet been validated for a sufficiently diverse set of aquatic organisms and endpoints, coupled with the full range of water quality conditions. . . . the BLM could have current practical applications . . . as an alternative or complementary method to the current water-effects ratio (WER) approach."

Pursuant to their review, the SAB made several recommendations with regard to the BLM before it could be used in developing site-specific criteria for silver (USEPA, 2000). Again, quoting from this review:

“Finally, the Committee provided recommendations for further research to provide additional validation in the following areas:

- a) Prediction of chronic and sub-acute toxicities not currently supported by the BLM.
- b) Broadening the supporting database to include greater taxonomic and functional diversity and additional comparisons with the water-effect ratio method.
- c) Gaining better mechanistic and kinetic understanding, i.e., distinguishing relative differences in binding affinity and toxicity mitigation among hardness cations (e.g., Ca, Mg, Mn) and other “biotic ligands” besides the fish gill, and evaluating predictability of the model under non-equilibrium water quality conditions.
- d) Distinguishing the events and kinetics of DOC complexing with divalent cations and biological uptake to improve interpretation’s of model predictions.
- e) Applicability to multiple metals, and sensitivity analyses for varying water chemistry conditions.”

In view of the need to ultimately incorporate the BLM into the criteria evaluation process, supplemental analyses have been performed since the time of the SAB report, in part to address SAB’s concerns and recommendations. This report provides an updated summary of the technical basis of the BLM for copper, with further descriptions of these updated analyses.

2.0 THEORETICAL BACKGROUND

2.1 Description of Generalized BLM Model Framework

The conceptual framework for the BLM is an adaptation of the Gill Surface Interaction Model, (GSIM) originally proposed by Pagenkopf (Pagenkopf, 1983) and more recently utilized by Playle and coworkers (Playle et al., 1992; Playle et al., 1993b; Janes and Playle, 1995; Hollis et al., 1996; Hollis et al., 1997; Playle, 1998; Richards and Playle, 1998; Wood, 1999), and the Free Ion Activity Model (FIAM), as previously reviewed by Morel (Morel, 1983; Morel, 1993) and Campbell (1995). The generalized framework is illustrated in Figure 1. The model is based on the hypothesis that toxicity is not simply related to total aqueous metal concentration, but that both metal-ligand complexation and metal interaction with competing cations at the site of action of toxicity need to be considered (Pagenkopf, 1983; Meyer et al., 1999). Mortality occurs when the concentration of metal bound to the biotic ligand exceeds a threshold concentration.

The BLM simply replaces the fish gill as the site of action with a more generally characterized site, the biotic ligand. The reason for this replacement is to emphasize that this model should be applicable to other aquatic organisms for which the site of action is not readily accessible to direct

measurement. In fact, it is likely that these principles should apply to any organism for which metal toxicity is associated with a discrete site of action and when the chemical speciation of the metal has an effect on availability of the metal to the biological receptor.

The role of metal complexation is critical, because formation of organic and inorganic metal complexes renders a significant fraction of the total metal less bioavailable. In fact, this modeling framework provides an operational definition for the expression “bioavailability of metals” (Meyer 2002a). As shown in Figure 1, dissolved metal exists in solution partially as free metal ion. This species is hypothesized to be the toxic species in more simplified versions of the free ion activity model of toxicity. The inorganic and organic complexes may or may not directly contribute to toxicity. The bioavailability of these compounds is inferred from experimental observations of changes in metal toxicity with changing chemistry. It is for this reason that we concentrate on comparing predicted and observed toxicity as the test of the model's utility.

The toxicity of metals to organisms is assumed to occur as the result of metal reacting with the physiologically active binding sites at the site of action resulting in the formation of a metal-biotic ligand complex (Figure 1, right-hand side). For fish, the biotic ligand appears to correspond to sites on the surface membrane of the gill responsible for regulating sodium ion uptake (McDonald et al., 1989). In the BLM metal ions or complexes may bind to the biotic ligand as well as other cations (e.g., Ca^{2+} , Na^+ , and H^+ ; Figure 1). As a result, the presence of these cations in solution can mitigate toxicity, with the degree of mitigation depending on their concentrations and on their strength of binding to the biotic ligand.

2.2 Model Formulation

The BLM is based on the hypothesis that the metal-biotic ligand interaction can be represented in the same way as any other reaction of a metal species with an organic or inorganic ligand. Consider a ligand L_b^- , in this case the biotic ligand, and a divalent metal cation M_i^{2+} . The charge on the biotic ligand is unknown. We assign a negative charge to be definite because it binds positive cations. However, this choice has no practical significance. The concentration of the metal-biotic ligand complex, $M_iL_b^+$, is determined by the mass action equation

$$[M_iL_b^+] = K_{MiLb}[M_i^{2+}][L_b^-] \quad (1)$$

where K_{MiLb} is the stability constant for the metal-ligand complex and the square brackets denote molar concentration. It is assumed that protonation can also occur with the formation of a proton-biotic ligand complex HL_b with concentration $[HL_b]$ and stability constant K_{HLb} .

$$[HL_b] = K_{HLb}[H^+][L_b^-] \quad (2)$$

The mass balance equation associated with the biotic ligand L_b^- is

$$[L_b^-]_T = [L_b^-] + [HL_b] + \sum_{i=1}^{NMi} [M_iL_b^+] \quad (3)$$

where $[L_b^-]_T$ is the total binding site density of the biotic ligand (e.g., nmol of available sites/g of tissue), $[HL_b]$ is the concentration of protonated sites, and N_{Mi} is the number of metal complexes $M_iL_b^+$ e.g., CuL_b^+ , CaL_b^+ , etc., that form with the biotic ligand L_b^- .

The analogous equations for the metal cation M_i^{2+} and the other aqueous ligands L_j^- that form metal-ligand complexes are

$$[M_iL_j^+] = K_{MiL_j}[M_i^{2+}][L_j^-] \quad (4)$$

$$[HL_j] = K_{HL_j}[H^+][L_j^-] \quad (5)$$

$$[L_j^-]_T = [L_j^-] + [HL_j] + \sum_{i=1}^{N_{Mi}} [M_iL_j^+] \quad (6)$$

where K_{MiL_j} and K_{HL_j} are the stability constants for these ligands. The mass-balance equations for the metal cations in the solution are

$$[M_i^{2+}]_T = [M_i^{2+}] + [M_iL_b^+] + \sum_{j=1}^{N_{Lj}} [M_iL_j^+] \quad (7)$$

where N_{Lj} is the number of metal-ligand complexes, including hydroxyl complexes, that M_i^{2+} forms.

In the BLM it is assumed that the quantity of metal bound to the biotic ligand $[M_iL_b^+]$ is insignificant relative to the aqueous species (Morel, 1983; Meyer, 1999a). Therefore Equation 7 becomes

$$[M_i^{2+}]_T = [M_i^{2+}] + \sum_{j=1}^{N_{Lj}} [M_iL_j^+] \quad (8)$$

The aqueous chemistry speciation equations (Equations 4-6, 8) can be solved as discussed below. Then the biotic ligand equations (Equations 1-3) can be evaluated. This convenient approach is adopted below.

The parameters required that are specific to the BLM are the conditional stability constants, K_{HLb} and K_{MiLb} $i = 1, \dots, N_{Mi}$ for the proton- and metal- biotic ligand complexes (Equations 1-2), and the total site density $[L_j^-]_T$ (Equation 3). For fish, the site densities and stability constants are determined on the basis of experimental fish gill measurements. These are currently available for a number of metals, including copper, cadmium (Playle et al., 1993a; Playle et al., 1993b) and silver (Janes and Playle, 1995). For other organisms, the values are obtained by fitting the model to observed mortality data, as discussed below. The relevant mole balance equations and species formation reactions and related constants that apply to the copper BLM model are summarized in Appendix A.

The model is based on the idea that mortality (or other toxic effect) occurs if the concentration of metal on the biotic ligand reaches a critical concentration C_{Mi}^* .

$$C_{Mi}^* = [M_iL_b^+] \quad (9)$$

This critical concentration for mortality can only be determined from toxicity experiments that establish the LC50 (or EC50) concentrations for a variety of toxic metal and competing cation concentrations. Once the site densities and stability constants are known, the critical concentration

can be determined by computing the biotic ligand concentration corresponding to the aqueous LC50 concentration. The validity of the BLM can be established only if the critical concentration C_{Mi}^* is the same over the entire range of water chemistry tested. Examples of this analysis will be given below. This critical concentration is referred to as the “LA50”, or the lethal accumulation of metal on the biotic ligand associated with 50% mortality (Meyer et al, 1999b; Meyer et al., 2002).

2.2.1 Chemical Model- Inorganic Metal Speciation

The chemical speciation computations are standard and may be performed with any of several models that exist: for example, MINEQL (Westall et al., 1976), MINTEQA2 (Brown and Allison, 1987; Allison et al., 1991), or the program used for the computations presented below, CHESS (CHemical Equilibria in Soils and Solutions; Santore and Driscoll, 1995). The inorganic speciation is the straightforward part of the computation because the ligands are well characterized, for the most part, and their binding constants are known (e.g., Smith and Martel, 1993). The difficult part is modeling the complexation of metal cations by organic matter. Although Pagenkopf (1983) recognized the ameliorating effect of organic matter on toxicity, the effect of organic matter was neglected in his original model formulation. It was applied to test results using laboratory waters with low organic matter content. However, dissolved organic matter is known to be an important ligand for most metals in most natural waters.

2.2.2 Chemical Model- Dissolved Organic Matter Complexation

There is no shortage of models that have been proposed for modeling the complexation of metals to dissolved and particulate organic matter; e.g., models by Van Riemsdijk and colleagues (Benedetti et al., 1995; Kinniburgh et al., 1996) being a recent example of a comprehensive modeling framework. For many of these models, the parameters that have been estimated apply to a specific experimental data set. What is required is a model that has been calibrated to multiple data sets, and for as many metals as possible.

Since this is an important criterion, Version V of the Windermere Humic Aqueous Model (WHAM), developed by Tipping and coworkers (Tipping and Hurley, 1992; Tipping, 1993) has been selected for use. The model is fully described and the computer code is available (Tipping, 1994). It contains a detailed model of proton binding. This is then expanded to include metal cation binding. The idea is that the proton and the metal cations are competing for the same sites, so a detailed model of proton binding is the essential first step.

This description follows that provided by Tipping and co-workers (Tipping and Hurley, 1992; Tipping, 1993). Protons bind to carboxyl (type A) and phenolic (type B) sites. A uniform distribution of pK s is specified for each type of site for which $pK = -\log_{10}K$ and K is the stability constant for the proton-site binding reaction. The site distributions are parameterized by the median pK_A and pK_B and their ranges ΔpK_A and ΔpK_B . Each uniform distribution is approximated using four discrete pK 's for each type: K_{H1}, \dots, K_{H4} and K_{H5}, \dots, K_{H8} , respectively, where

$$pK_{H1} = pK_A - \Delta pK_A/2 \quad (10)$$

$$pK_{H2} = pK_A - \Delta pK_A/6 \quad (11)$$

$$pK_{H3} = pK_A + \Delta pK_A/6 \quad (12)$$

$$pK_{H4} = pK_A + \Delta pK_A/2 \quad (13)$$

and the analogous equations for pK_{H5}, \dots, pK_{H8} . The site density of type A sites is n_A . Because humic and fulvic acids have fewer B sites, their site density is assumed to be $n_B = n_A/2$. The electrostatic interactions are modeled using an empirical formulation with one adjustable parameter, P , which is related to the surface complexation model formulations (Westall and Hohl, 1980; Dzombak and Morel, 1990). Tipping and co-workers have fit this model to various sets of acid-base titrations of organic matter to determine the six model parameters: n_A , the pK 's and ΔpK 's, and the electrostatic parameter P .

The stability constants for metal binding are parameterized by only two additional constants. This is a remarkably parsimonious construct. The idea is to specify the metal binding constants using the proton binding pK 's. The metal stability constants, K_{Mi} , $i=1, \dots, 8$ are defined relative to the proton binding constants K_i via two parameters: K_{MA} and K_{MB}

$$K_{Mi} = K_{MA}/K_{Hi} \quad i = 1, \dots, 4 \quad (14)$$

$$K_{Mi} = K_{MB}/K_{Hi} \quad i = 5, \dots, 8 \quad (15)$$

where the K_{Mi} are the discrete metal stability constants analogous to the K_{Hi} proton binding stability constants. In addition to binding to the proton sites individually, binding to two sites at once (bidentate sites) is also allowed. The binding constants for these sites K_{Mij} are computed from the product of the metal monodentate binding constants

$$K_{Mij} = K_{Mi}K_{Mj} \quad (16)$$

For the sake of simplicity, only 11 of the possible pairs are used. From data fitting, Tipping and Hurley (1992) developed the following relationship between K_{MA} and K_{MB} :

$$pK_{MB} = 1.38 pK_{MA} + 2.57 \quad (17)$$

Use of this relationship reduces the number of metal specific parameters to one. This is the most parsimonious parameterization possible. For each metal, only one parameter K_{MA} is required. The rest follow from the equations given above (Equations 14-17).

The status of the level of calibration of Version V of WHAM will be summarized in Section 3. The model equations and parameter values, as employed herein for copper, are summarized in Appendix A.

2.3 Relationship of Copper Accumulation to Acute Toxicity

To date, the BLM has been used to describe the toxicity of copper to freshwater fish such as fathead

minnows (*Pimephales promelas*) and rainbow trout (*Oncorhynchus mykiss*) and invertebrates such as *Daphnia magna*, *Daphnia pulex*, *Ceriodaphnia dubia*, *Hyallela azteca*, *Lumbriculus variegatus*, and *Mytilus edulis*. Due to the relative ease with which gill tissue can be excised for the measurement of accumulated metals, information on metal binding to gill membranes that has been used for all organisms has come from fathead minnow and rainbow trout. These species have comparable sensitivities to copper and have been studied extensively (USEPA, 1985). Several studies have shown that when juvenile fathead minnows and rainbow trout are exposed to copper, there is a relatively rapid increase above background levels of copper bound to the gill. Playle and co-workers, working with fathead minnows, showed that this rapid initial increase takes place over a time scale of a few hours to a day (Playle et al., 1992). Similar data for juvenile rainbow trout indicates that this rapid initial increase in gill copper is followed by a more gradual, longer term, increase (MacRae et al., 1999). It is believed that the rapid initial increase in gill copper reflects binding to physiologically active receptor sites at the gill surface. More specifically, the accumulation reflects, at least in part, an interaction of copper with Na-K ATPase, an enzyme that is essential for the proper functioning of the iono-regulatory control processes of fish. For acute toxicity then, this enzyme is considered to be the biotic ligand for fish, and it is associated with the gill. Hence, it is necessary to predict metal accumulation at the gill surface, presumably metal that is associated with this enzyme, to be able to predict metal toxicity to fish.

If the approach is to be viable, it is necessary that there be a relationship between gill accumulation and mortality. Information on the amount of copper bound to gills that will result in lethality comes from determinations of copper toxicity and mortality in juvenile rainbow trout (MacRae, 1994; MacRae et al., 1999). Juvenile (15-40 g) rainbow trout (*Oncorhynchus mykiss*) were exposed to sub-lethal copper concentrations for 24 hours. The total copper concentrations were kept constant (10 ug/L) for this series of exposures, but the copper activity was varied by adding different organic ligands, with varying affinities for copper, to the test water. After 24 hours, individual fish were removed from the water, their gills were excised, and copper accumulation was determined. As a result, measured gill accumulation was shown to be related to copper activity.

In a parallel set of exposures with replicated chemical conditions, a 120-hour LC50 was determined. A plot of 24-hour gill accumulation versus 120-hour LC50 yields a dose-response relationship based on gill copper (Figure 2). As shown, the gill copper LC50 -- the total copper concentration on the gill that causes 50% mortality -- is estimated to be 22 nmol/gram wet weight (nmol/g_w). There is a background gill copper concentration -- the concentration associated with control-level mortality (i.e., < about 25%) -- of approximately 12 nmol/g_w. This compares well with the fathead minnow background gill copper level, approximately 12 nmol/g_w, that was measured by Playle and co-workers (1992) in the absence of added copper. Based on these results, the gill copper LA50 should be approximately 10 nmol/g_w -- the gill Cu LC50 of 22 nmol/g_w minus the background concentration of 12 nmol/g_w. Although subsequent analyses of toxicity data (Section 3) have led to a refined estimate of the gill Cu LA50, acceptance of the existence of a cause-effect relationship such as that shown by the data of Figure 2, one that is invariant of water chemistry, is a fundamental premise upon which the BLM is based. A similar result has been demonstrated for the toxicity of copper to an oligochaete worm (*Lumbriculus variegatus*), for which the copper LA50 was approximately, 0.17-0.34 umol Cu/g dry wt. (Meyer et al., 2002). While in a strict sense, water chemistry is known to

have additional physiological effects upon the organism, the idea that there exists a unique level of accumulation at the biotic ligand that is associated with a fixed effect is a significant step forward in comparison to a hardness-based WQC, at least with regard to being able to predict the degree of effect that is associated with alternative exposure conditions.

2.4 Description of Species on the Biotic Ligand

As noted previously, the biotic ligand in fish is associated with the gill and is considered to be Na,K-ATPase, an enzyme that has an important role in the regulation of sodium levels not only in fish, but in essentially all forms of aquatic life. Cu and other metals will interact with this enzyme, with the level of complexation of the metal-biotic ligand being related to the degree of effect, that being an inhibition of the active uptake of sodium in freshwater organisms (or active excretion of sodium in saltwater organisms). Because of the need to evaluate the level of accumulation of the available toxic metal species at the biotic ligand, it is introduced as an additional biotic component that must be considered in the mass balance of substances in the chemical model. The total site density is set at 30 nanomoles per gram wet weight of gill (30 nmol/g_w)

The principal copper species that is assumed to result in adverse effects is often considered to be Cu²⁺, but it need not be limited to Cu²⁺ only, and other species such as CuOH⁺ may also bind to the biotic ligand and result in adverse effects as well. The bioavailability of CuOH⁺ has been implicated in other studies (Chakoumakos et al., 1979; Pagenkopf, 1983; Brown and Markich, 2000). It is known that other cations in solution will also affect and to some degree mitigate the toxicity of metals such as copper. In the context of the biotic ligand model, the mechanism of this protective effect is represented as a competitive binding effect, whereby another cation, such as Ca²⁺ or H⁺ or Na⁺, can decrease the binding of copper to the biotic ligand. This decreases the accumulation of copper, thereby decreasing the degree of effect on the organism. Hence, the increase in the dissolved copper LC50 in the presence of increasing concentrations of competing cations. Although other physiological mechanisms might also be partially responsible for observed mitigation of toxicity (e.g., the effect of sodium on sodium uptake kinetics and of calcium on membrane permeability and, thus, on the sodium loss rate), the use of competitive cation interactions with the biotic ligand offers an expeditious way to account for these effects in the context of the well-established framework of a chemical equilibrium model.

The cation-gill complexes that are considered in the model at this time are Cu²⁺ and CuOH⁺, the metal species that are associated with adverse effects, and Ca²⁺, H⁺ and Na⁺, the competing cations that mitigate toxicity. The total concentrations of each of the chemical components that are included in the model are present in the form of a variety of chemical species. The sum of the concentrations of all of these species, with the appropriate stoichiometric relationships applied, must equal the total concentration of the chemical component. These mole balance relationships are listed in Appendix A1.

2.5 Alternative Computational Modes

The BLM uses chemical speciation to determine the distribution and chemical activity of the metal in site waters. Chemical speciation calculations, therefore, are an important component of the model predictions. However, unlike traditional metal speciation calculations where a known (usually measured) quantity of metal is used, the BLM is used to determine an unknown quantity of metal that would correspond to some toxicological effect; the effect might be mortality to a single species, or an acute or chronic criterion derived from a species sensitivity distribution. The BLM, therefore, does not need to know the amount of metal in the test water, and although the metal quantity can be input to the model, this quantity is not used in the speciation calculations, but will be used to compare to the model predicted criteria values to check for impairment. The method used to perform each of these tasks is described in further detail below.

2.5.1 Metal Speciation Computations

First, chemical components are defined as a subset of all available chemical species. Components must be selected such that no component can be formed as the product of a reaction involving only other components, and all remaining species can be defined as the product of a reaction involving only components. From these components, a generic set of equations that describe the chemical equilibrium system can be summarized following the conventions described in Santore and Driscoll (1995). A set of mole balance equations is defined for the chemical components to account for all chemical species in the equilibrium system.

$$T_j = \sum_i S_i a_{ij}$$

Where the concentration of an individual chemical species is represented by S_i and the stoichiometric coefficient representing the contribution of species S_i to mole balance T_j is indicated by a_{ij} .

The species concentrations can be expressed as a function of the concentrations of each of the chemical components by the use of mass action expressions. The mass action expressions for the formation of each of the species in the BLM are listed in Appendix A2. These expressions include specification of the interactions at the gill that will be needed for evaluating the accumulation and, hence, toxicity of copper to fathead minnow (*Pimephales promelas*). For each reaction, the concentration of a species i , S_i , can be calculated as:

$$S_i = K_i \prod_k C_k a_{ik}$$

where the stoichiometric coefficient between species i and component k (a_{ik}) and the K_i for each reaction are known (Appendix A2), and the concentration of each component C_k is an unknown. The values of K_i in Appendix A2 are modified for the specific conditions of ionic strength and temperature. Ionic strength corrections can be provided by the extended Debye-Huckel Equation (Morel, 1983) for inorganic species, and by a Donnan-layer expression for organic species (Tipping, 1994). Substituting these mass-action expressions into the mole-balance equations generates a system of 26 equations (T) in 26 unknowns (C). This system of equations can be solved simultaneously to derive the final chemical distribution at equilibrium.

2.5.2 Metal Toxicity Computations

When the BLM is used to predict toxicity, the dissolved copper concentration is not known *a priori*. In fact, the primary utility of the BLM is that it provides a computational basis for predicting the amount of dissolved metal that will be toxic to an organism. The BLM adapts the above chemical speciation calculation to predict toxicity by considering the critical accumulation or LA50 associated with a given toxicological endpoint for a given organism. In this case, the total metal concentration is determined such that the sum of all toxic metal species bound to the biotic ligand equals the LA50. These relationships are used to extrapolate the final acute value derived from the species sensitivity distribution to a site water. The result is an estimation of what a final acute value would have been, if the entire species sensitivity distribution had been measured in the site water. This extrapolation has been shown to reasonably predict the effects of local water chemistry parameters (such as pH, DOC, alkalinity, Ca concentrations, etc) on metal toxicity. Although metal accumulation on the biotic ligand is a means to estimate these effects, the predicted accumulation of metal on the biotic ligand is not the primary goal of the application of the BLM.

3.0 MODEL CALIBRATION

This section summarizes the sources of information and the data and modeling analyses that have been performed to arrive at the current calibration of the BLM. Information will first be summarized with regard to both the inorganic and organic speciation sub-models. The application of the model to accumulation and toxicity data will also be presented.

3.1 Sources of Thermodynamic Information

3.1.1 Inorganic Metal Speciation

Inorganic metal speciation requires the specification of the total concentrations of the inorganic components that are input to the model. This information is typically assigned on the basis of measurements that are made for the water sample of interest. The log K's are typically set on the basis of the NIST database, though in the case of the BLM, where WHAM is used, the log K's that have been used by Tipping are employed. Some exceptions to log K's that are reported in the NIST database are therefore used. This is considered to be a reasonable approach, in light of the good agreement that has been achieved in the application of WHAM to datasets that have been used to characterize copper-organic matter interactions. The calibration of the organic binding parameters will depend, to some extent, on the values of the inorganic stability constants used when WHAM was developed. Values for log K's that are used in the model are summarized in Appendix A.

3.1.2 Interactions of Copper with Organic Matter

As stated previously, the approach that is employed by the BLM to evaluate the complexation of copper by organic matter is based on Version V of WHAM. The formulation and structure of this model was presented previously in Section 2. Briefly, competing reactions are simulated as simultaneous equilibrium reactions, and equilibrium metal speciation includes formation of inorganic and organic complexes (Table 1). The WHAM-based formulation includes a complex description of reactive functional groups that are designed to emulate a continuous distribution of sites on humic and fulvic acids. Organic complexation includes a proton-dependent description of net molecular charge. The molecular charge is used to calculate an electrostatic adjustment to chemical binding of metals. Non-specific ion binding is simulated using a Donnan-type double layer model. Model V and WHAM have been previously calibrated with a number of metals and sources of dissolved organic matter. Given this prior extensive development effort by Tipping and co-workers (Tipping and Hurley, 1992 and Tipping, 1993), the model input parameters related to metal:organic matter interactions that are used in the BLM are consistent with Version V of WHAM. A brief overview of the results of this calibration effort is provided here.

Figure 3 presents a selection of the titration data used to evaluate the requisite WHAM parameters. Each graph, redrawn from Tipping and Hurley (1992), utilizes the following convention. The y-axis variable v is the concentration of metal bound to DOC (mol/g DOC) and the x-axis variable is the free metal concentration (mol/L). Both are plotted as $-\log$ of the concentrations following the

chemist's convention for $pH = -\log_{10}[H^+]$. Thus, small concentrations correspond to large numerical values. In order to retain the usual sense that increasing concentration corresponds to increasing distance from the origin, the numerical values decrease with increasing distance from the origin.

Figures 3A-3C present the data for copper. Figure 3A illustrates the effect of increasing ionic strength ($I = 0.001\text{ M}$ to 0.01 M). Increasing the ionic strength slightly reduces the amount of copper complexed to DOC. Figure 3B illustrates the effect of calcium competition ($[Ca] = 0\text{ M}$, 0.001 M and 0.01 M) on copper binding. Increasing the calcium concentration decreases the quantity of complexed copper because Ca^{2+} competes with Cu^{2+} for the same binding sites. Since calcium also competes with copper at the biotic ligand, the proper modeling of this competition for DOC sites is important. Figure 3C illustrates the effect of $pH = 5.14$, 7.00 , and 8.44 . As the pH decreases the increasing concentration of H^+ competes with Cu^{2+} for the binding sites and less copper is complexed to the DOC. The deviations at the more highly complexed copper concentrations are of less concern in this application since they are usually above toxic concentrations (i.e., $[Cu] \sim 10^{-9}\text{ M} = 0.06\text{ ug Cu/L}$, which is well below most acute LC50s for $[Cu_{total}]$. But it would be more appropriate for $[Cu^{2+}]$). The low concentrations are important. Figures 3D-3F present the effect of pH on the complexation of calcium, cadmium and lead. As with copper, less metal binds to DOC at lower pH levels. The careful and complete calibration of the WHAM model, illustrated in Figure 3, was the principal reason for its selection for use in this application.

The description of dissolved organic carbon includes humic and fulvic molecules. Adjusting the percent of organic matter in humic and fulvic forms can be used to specify variation in organic matter chemistry. Inorganic speciation includes aqueous hydroxide and carbonate complexes (Table 1). Although the chemical description of dissolved organic matter comes from Model V and WHAM, all equilibrium speciation reactions are simulated by CHESS.

In view of the preceding results, it appears that WHAM provides a robust model for the most complex aspect of the chemical speciation calculation: the complexation of metals to organic matter. Nevertheless, it is expected that further refinements will be incorporated into the model in the future, particularly at lower metal concentrations where even stronger binding sites may need to be included. This is almost certainly the case for low concentrations of silver, for which binding to sulfur-containing ligands has been found to be important (Bell and Kramer, 1999). Studies are currently in progress, both in the United States and in Europe, to obtain additional data that can be used to further refine the representation of copper interactions with organic matter. The BLM itself is of sufficient flexibility that refinements will be able to be readily incorporated into the chemical model, at such time in the future when it is appropriate to do so.

3.2 Calibration of the BLM to Copper Accumulation at the Biotic Ligand

A variation of the generalized BLM framework diagram that was presented previously (Figure 1) is shown on Figure 4, as it applies to copper. Pursuant to the BLM methodology, acute copper toxicity is directly related to a predetermined level of copper accumulation at the biotic ligand. By specifying the chemical characteristics of this biotic ligand and evaluating the concentration of copper accumulation associated with it, it is possible to predict the dissolved copper level that will be

associated with an prescribed effect, such as 50% mortality. What needs to be determined then are the values of model parameters that can be used to evaluate the level of accumulation at the biotic ligand.

As described in Section 2, acute metal toxicity in freshwater fish has been associated with the disruption of Na ion regulation (Playle et al., 1993a). Accumulation of copper at the gills of freshwater fish has been shown to inhibit Na ion influx and reduce Na-K ATPase activity (Playle et al., 1993b). For freshwater fish the biotic ligand associated with acute copper toxicity, therefore, is assumed to involve the transport mechanisms for Na ion regulation in the gill. Analytical techniques for measuring specific metal adsorption on the biotic ligand have not been developed. However, the adsorption of copper on gill surfaces has been measured over a wide range of water quality conditions (Playle et al., 1992; Playle et al., 1993a and 1993b). For this study, metal accumulation on the gill is assumed to be a surrogate measurement for specific accumulation on the biotic ligand.

The biotic ligand site density and binding constants for copper and other cations to the biotic ligand were originally derived from the experimental investigations of Playle and co-workers (1992 and 1993a). The amount of copper adsorbed to fathead minnow (*Pimephales promelas*) gills, over a range of total copper concentrations, was measured in these investigations and these measurements used to evaluate the binding site density and stability constants for copper adsorption (Playle et al., 1993b). This gill-binding model for fathead minnows was adopted for use in the present analysis. However, this model did not include binding of Na (Playle et al., 1993b). Evidence from copper toxicity determinations suggests that Na also has a protective effect (Erickson et al., 1987 and 1996) so binding of Na was evaluated and added to the fathead minnow database for the BLM for copper.

Using these parameter values and application of the BLM to predict Cu accumulation in fathead minnow gills results in good agreement with gill accumulation levels measured by Playle and coworkers (1993a; Figure 5). Although there is a considerable scatter of the data about the simulated line of fit, the overall trend in the data is reproduced by the model. For these data only the accumulation above the background gill Cu loading (12 nmol/g_w) that is associated with pristine conditions is shown. Copper binding shows a typical Langmuir-type adsorption pattern with an assumed maximum binding site density of 30 nmol/g_e in excess of background. These initial estimates of the biotic ligand binding constants have been adjusted in some cases on the basis of the calibration of the model to the results of toxicity studies, as described subsequently.

3.3 Response of the BLM to Variation in Hardness, DOC and pH

The next application of the BLM will be made using a very complete and well-characterized set of experiments by Erickson and co-workers (1987 and 1996) who investigated copper toxicity to larval fathead minnows. Fish were exposed to increasing concentrations of copper in these experiments and the LC50s were determined. Systematic variations of important water quality characteristics were employed to produce LC50s as a function of the concentrations of these variables. Three sets of experimental results will be used to illustrate the effects of dissolved organic carbon (DOC), hardness and pH on model performance, specifically, the prediction of biotic ligand accumulation levels. (These same data will also be presented subsequently, where the model is used to predict

toxic effect levels.) The thermodynamic constants used for WHAM, the gill/biotic ligand computations, and the base water chemistry are listed in Tables 1, 2 and 3, respectively.

3.3.1 Effect of Dissolved Organic Carbon on Copper Accumulation and Toxicity

The effect on copper LC50 of variations in DOC concentration is shown in Figure 6. The pH and hardness were held approximately constant for these experiments. The added DOC is Alrich humic acid. The results of Figure 6A demonstrate that the measured total Cu LC50 (filled data points and associated trend line) increases with increasing DOC concentration, as is often observed. This is rationalized in the model by assuming that the copper that forms a complex with DOC is not bioavailable. As a result, as DOC increases more copper is needed to exert the same degree of toxicity. Figure 6B shows the corresponding free copper (Cu^{2+}) LC50s. These LC50s were calculated using WHAM (V) and the reported chemistry and the total copper LC50's in Figure 6A. The results are somewhat variable, but they are approximately independent of DOC concentration. This is consistent with the free ion activity model of toxicity, where toxicity is directly related to the concentration (actually the activity) of free copper. Figure 6C presents the calculated gill copper concentrations associated with the measured total copper LC50 data. The gill copper concentrations were calculated using WHAM to compute the metal-humic acid complexes, and CHESS to compute the gill accumulation. The Cu^{2+} -gill, Ca^{2+} -gill and H^{+} -gill conditional stability constants and gill site density estimated by Playle et al. (1993) from measured gill copper concentrations were used. The exchangeable gill copper LA50 averages slightly less than 5 nmol/gw. This level of fathead minnow gill copper accumulation is a factor of two lower than the measured exchangeable gill copper LA50 for rainbow trout of 10 nmol/gw (Figure 2), which is quite encouraging. The fact that the gill copper LA50 is approximately constant across the DOC range tested indicates that, as was the case with free copper, gill copper concentration can also be used to predict acute mortality when DOC is varied.

3.3.2 Effect of Hardness on Copper Accumulation and Toxicity

The effect of variations in calcium concentration on copper LC50 is shown in Figure 7. The experiments were performed with DOC and pH held approximately constant. Over the range of experimental conditions of 0.5 to 2.5 meq Ca/L, the ratio of Ca:Mg was approximately 2:1 and the corresponding hardness range was 75 to 375 mg CaCO_3 /L. The data are displayed as before except that the LC50 results are plotted versus calcium. As was the case with increasing DOC, the measured total Cu LC50 increases with increasing Ca concentration (Figure 7A). This increase in LC50 with hardness is qualitatively consistent with the current water quality criteria (WQC) for copper, which increases as a function of hardness as well (USEPA, 1985). However, as discussed below, the magnitude is significantly less than expected.

Free copper LC50s corresponding to the total copper LC50s are shown in Figure 7B. Like the total copper LC50s, the free copper LC50s varied over the range of calcium levels tested. Therefore, a single free copper concentration is no longer uniquely associated with 50% mortality. This is not unexpected because copper, a cation, does not form complexes with calcium, another cation. Therefore, except for a relatively minor ionic strength effect (see Figure 3A), there is no interaction of calcium with copper in the water. Note that, in combination with the results from the DOC

experiments shown in Figure 6B, these results indicate that there is more than a 10-fold range in variation of free copper concentration, from less than 0.015 to 0.15 $\mu\text{mol/L}$, associated with the fathead minnow free copper LC50. These results indicate that considering only the free ion as the bioavailable ligand cannot be used to rationalize these data.

In contrast to the free copper results, the calculated gill Cu concentrations shown in Figure 7C indicate a relatively consistent concentration for the range of calcium concentrations tested. The average fathead minnow gill copper concentration, 12 nmol/gw, is in reasonably good agreement with the gill copper concentration of slightly less than 5 nmol/gw calculated for the DOC experiments (Figure 6C) and with the gill Cu LA50 of about 10 nmol/gw of Figure 2. The reason that the gill copper tends to be relatively constant, even as the free copper concentration increases, is that the calcium competes with the free copper for binding at the biotic ligand on the gill. Hence, a higher free copper concentration is required to achieve the same gill Cu concentration associated with mortality.

It is interesting to note that for the calcium experiments the observed increase in total copper LC50 -- slightly more than a factor of two over the range of conditions tested -- is half as much as is expected from the hardness correction incorporated in the current copper WQC -- 4.5-fold, for hardness increasing from 75 to 375 mg CaCO_3/L (USEPA, 1985). The BLM framework provides an explanation for this difference (Meyer, 1999). The experiments by Erickson (1996) with varying hardness were conducted with constant alkalinity, by adding calcium in the form of CaSO_4 . Thus, as hardness is increased in these experiments, the only factor that mitigates toxicity is competition between Cu^{2+} and Ca^{2+} for the gill sites. In contrast, for most studies from which the WQC is based, the hardness was adjusted by adding CaCO_3 to the water. As a result, bicarbonate alkalinity increased as well. The added CO_3^{2-} reacts with Cu^{2+} to form CuHCO_3^+ and CuCO_3^0 . The resulting "hardness" effect is actually a calcium carbonate effect (Meyer, 1999). Hence toxicity is mitigated to a greater degree than in the experiments by Erickson et al. (1996) in which the sulfate ion had no effect. This explains the smaller than expected effect of calcium hardness on copper LC50.

3.3.3 Effect of pH on Copper Accumulation and Toxicity

The effect of changes in pH on total copper LC50 is shown in Figure 8A. The total copper LC50 increases from about 0.1 to 2 $\mu\text{mol/L}$ as the pH increases from 6.5 to 8.8. In addition to the total Cu concentrations, the free copper ion activity was also measured with a selective ion electrode. It is fortunate that Erickson (1996) made these measurements. When the concentration of total Cu is used to predict the free copper in solution using WHAM, the computed results for Cu activity were significantly less than the measured data. The apparent difficulty, which is limited to this set of data only, and its solution are discussed in Section 3.4.3. However, the measured free copper activity, shown in Figure 8B, can be used to compute gill copper concentrations, which are shown in Figure 8C. The variation in predicted gill copper concentrations reflects the variation in the cupric ion activity measurements. The average gill copper concentration of approximately 7 nmol/g_w is within the range of the results for the two previous sets of experiments in which DOC and calcium were varied. The slight decreasing trend of gill Cu with increasing pH might be explained in part by the provision for CuOH^+ binding to the biotic ligand. This effect is not included in the calculations,

presented thus far, but if it was accounted for, the pH response would be flattened. Results shown on the two previous plots would not be significantly impacted, since the pH was generally lower, and thus CuOH^+ was also present at a lower concentration.

In the context of the BLM framework, pH affects copper toxicity in several ways. First, the model predicts that toxicity will decrease with increasing pH as a result of the effect of pH on speciation and complexation of copper. As pH increases, the fraction of Cu that exists as copper carbonate complexes increases, thereby reducing toxicity. Further, the deprotonation of DOC at higher pH levels increases the degree to which the copper-DOC complex forms, which reduces bioavailability as well. These effects on toxicity are offset to some degree by the competition between the H^+ and Cu^{2+} ions binding to the biotic ligand, a factor that by itself would result in an increase in toxicity as pH increases (i.e., as the H^+ concentration decreases).

However, there is an indication in Figure 8B that, at the LC50, free copper might actually be decreasing with increasing pH above pH 8.0. Reduced free copper concentrations at high pH when total copper equals the LC50 would indicate the copper is more toxic at high pH than complexation reactions would predict. If we compare predicted copper LC50 based on the BLM formulation assuming only free copper is bioavailable with measured copper LC50 at high pH, it is clear that the BLM does predict that copper is less toxic at high pH than measured LC50 values indicate (Figure 9). Agreement between measured and predicted copper LC50 seems to be good at pH values below 8.0, but at pH values above 8.0 the model predicts LC50s that are too high. This discrepancy suggests that another copper species, in addition to free copper, is bioavailable and can exhibit toxic effects.

From a chemical standpoint, it is reasonable to suggest that additional copper species are capable of sorbing onto gill membranes. For example, the WHAM model assumes that both Cu^{2+} and CuOH^+ species bind to natural organic matter (Figure 10). At pH values below 8.2 to 8.5 (depending on the total metal concentration) the predominant form of copper bound to NOM is the free copper ion. However, at high pH values CuOH^+ is the dominant form of copper bound to NOM. The gill surface may also bind Cu^{2+} and CuOH^+ in relatively similar amounts. Further support for this assumption comes from observations that the pH at which we would expect the predominant copper species to shift from free ion to copper hydroxide is exactly the pH at which the model, formulated only with bioavailable free copper, predicts reduced toxicity. Incorporating the adsorption of both Cu^{2+} and CuOH^+ onto gill surfaces can be accomplished by assuming relative binding strengths for these ions that are similar to what we would expect for adsorption onto natural organic matter. The result, shown in Figure 11, shows that at the LC50 we would expect to see predominately free copper bound to the biotic ligand at pH values below 8.5, and predominately CuOH^+ bound to the biotic ligand at pH values above 8.5. It was determined by comparison with toxicity data from Erickson et al., 1996 result that slightly reduced binding of CuOH^+ gave the closest approximation of the observed pH response which is why the pH at which the conversion from Cu^{2+} to CuOH^+ occurs at a slightly higher pH on the biotic ligand (Figure 11) than it does on natural organic matter (Figure 10). The resulting model that includes both Cu^{2+} and CuOH^+ bioavailable species matches the observed pH response much more closely (Figure 12).

3.3.4 Summary

The results presented above indicate that the BLM describes the variation in copper toxicity to fathead minnows. The calculated fathead minnow gill Cu LC50s, averaging 5 to 12 nmol/gw over the range of DOC, hardness and pH conditions tested, are in reasonable agreement (approximately \pm a factor of 2) with the reported rainbow trout exchangeable gill Cu LC50 (10 nmol/g_w, Figure 2). This finding is consistent with comparable sensitivity of fathead minnows and rainbow trout to copper (USEPA, 1985). It is also consistent with recent findings that fathead minnows and rainbow trout accumulate copper on their gills in a similar manner, and that binding constants for metal-gill interactions determined for one species can be generalized to other fish species (Hollis et al., 1997). The copper-gill pKs for rainbow trout (7.5) and brook trout (7.2) found by MacRae et al. (1999) are quite close to pK = 7.4 as determined by Playle et al. (1993a) for fathead minnows. These results indicate that the BLM can explicitly account for variation in toxicity resulting not only from changes in hardness, but from site-specific variations in DOC, pH and alkalinity as well.

3.4 Application of the BLM to Fish Toxicity Data

The BLM will next be applied in the analysis of 96-hour toxicity data for fathead minnow (Erickson et al., 1987 and 1996)

3.4.1 Relationship of Biotic Ligand Accumulation Level to Effect

Information on the amount of copper bound to gills associated with lethality, described previously in Section 2, is derived from the determinations of copper accumulation and mortality to juvenile rainbow trout (MacRae, 1994; MacRae et al., 1999). It was shown that approximately 10 nmol/g_w of copper at the gill is the lethal accumulation level that is associated with 50% mortality, the LA50. Ideally a similar experiment would be performed to determine a gill-Cu dose response relationship for other fish, including fathead minnows. Although the rainbow trout gill-Cu LC50 was determined for a different species and using a longer duration toxicity exposure, an LA50 of 10 nmol/g_w was used as an initial estimate of the Cu LA50 for fathead minnow. This value was later adjusted based on calibration to larval fathead minnow LC50 data (see section 3.4.3).

3.4.2 Evaluation of the Predicted LC50 Using the BLM

The LA50 is used in the BLM to predict the dissolved copper LC50 by evaluating the amount of dissolved metal that will result in this critical gill Cu accumulation. Conceptually, the LC50 determination can be thought of as a titration of copper on the gill in the test water. For example, accumulation of copper on the gill in response to copper additions in a clean laboratory water with low concentrations of DOM would result in rapid accumulation on the gill (Figure 13). From the point in the titration when gill-Cu equals the LA50, the dissolved copper LC50 can be determined by interpolation.

The BLM uses a root-finding approach to determine values of acute metal LC50. The model assumes an initial LC50 value, and this concentration is used to determine the amount of metal that

is bound to the gill. The calculated gill-metal concentration is compared to the critical value and a new approximation is determined with a higher or lower copper concentration as necessary. New approximations are determined by expanding the range of trial values using an exponentially expanding step size until the critical gill concentration has been bracketed. The LC50 value is then found iteratively, using false position to update trial values.

3.4.3 Application of the BLM to a Bioassay Dataset for Fathead Minnow With Water Treatments in Synthetic and Natural Waters

An excellent dataset is available for the development and testing of the ability of the BLM to predict copper toxicity over a range of water quality characteristics (Erickson et al., 1987 and 1996). In these experiments, acute copper toxicity (96-hour LC50) determinations were made for fathead minnow, in both static and flow-through exposures, and in natural and synthetic waters. The test conditions were manipulated so that the effects of changes in pH, DOC, cations (Ca^{2+} , Mg^{2+} , K^{+} , and Na^{+}), and anions (HCO_3^{-} , Cl^{-} , SO_4^{2-}) could be independently examined.

The BLM was originally used to predict acute copper toxicity (96-hour LC50) to fathead minnow using gill binding parameters as originally determined by Playle and coworkers (1993b) and the LA50 value from MacRae and coworkers (1999). However, the fathead minnow bioassay results (Erickson et al., 1987 and 1996) suggested that beneficial effects from Na additions were sufficient to justify modification of the gill binding model to include these effects. A provision for Na accumulation on the gill was incorporated in the BLM application. Log K values for Na-gill and Ca-gill binding were calibrated or refined using the Erickson et al. fathead minnow data (see Appendix B of Erickson et al., 1987 and Table 2 in Erickson et al., 1996). Refinement of the value of the copper LA50 for fathead minnow was also made on the basis of BLM analyses of these data. These analyses led to a determination that an LA50 of 5.48 nmol Cu/g_w, was best suited for use in predicting the 96-hour dissolved copper LC50 for fathead minnow.

An input dataset was constructed from measured water chemistry when available, or from nominal concentrations calculated from base water chemistry plus chemical additions when measured values were not available (Erickson et al., 1987). Concentrations of DOC were not measured by the authors in the exposure water, however a background DOC concentration of 1.0 mg C/L for exposures conducted in Lake Superior water and 0.1 mg C/L in the synthetic waters was assumed (personnel communication with R. Erickson). Background concentrations of dissolved organic carbon (DOC) were assumed to be composed of 10% humic acid and 90% fulvic acid. Additions of DOC made with Aldrich Humic Acid were assumed to be 100% humic acid.

Predictions of fathead minnow copper toxicity using the BLM were compared to measured LC50 values. Individual experiments reported by Erickson et al. (1987) were designed to test the effect of specific water quality adjustments on copper toxicity. Comparison of BLM predictions for all static test results in the fathead minnow dataset are shown in Figure 14. Shown in the figure is the line of perfect agreement (solid) and dashed lines showing plus or minus a factor of two for reference. Measured LC50 values cover a wide range from 10 to over 1000 ug/L due to effects of water chemistry on copper toxicity. Despite the wide range in toxicity, nearly all predicted values

are within a factor of 2 of measured values. For comparison with toxicity test results, agreement within a factor of two is quite good given the variability between replicate measurements. For example, the replicate Cu LC50 values measured in Lake Superior water by Erickson and co-workers (1987) range over a factor of six from lowest to highest (13 observations, minimum Cu LC50 28 ug/L and maximum of 172 ug/L). Note that several of the outlying points are comparisons of the model results to exposures with high K concentrations (plotted with open symbols). Erickson et al. found that elevated K resulted in an increase in copper toxicity. This effect has not been incorporated in the Cu BLM and therefore, the LC50 values that are predicted by the BLM are higher than the measured values for these exposures. With the exception of these results, the BLM can generally explain the effects of water chemistry on copper toxicity to fathead minnow.

Given the very good level of agreement that was observed between measured and predicted copper LC50 values in static test exposures, it was initially somewhat surprising to see that model could not predict the flowthrough test results. The explanation for this discrepancy is believed to be associated with the kinetics of copper complexation with natural organic matter. In a recent study by Ma et al. (1999), the cupric ion activity that was measured shortly after mixing copper with natural organic matter (NOM) was significantly greater than the activity that was measured following 24 hours of equilibration between the copper and the NOM. Toxicity tests with *Ceriodaphnia dubia* in flow-through bioassay chambers were sensitive to the equilibration time of copper and DOC mixtures used in the exposure chambers. Toxicity tests with 1-hour hydraulic residence times (HRTs) resulted in lower total Cu LC50s than tests with longer HRTs (Ma et al., 1999).

This kinetic information has important implications for how we design and interpret toxicity tests. If the kinetics of the binding reaction is estimated from the Ma et al. (1999) data, we can simulate the time series of free copper under various test conditions. For a static test where the metal and sample containing NOM are mixed just before the start of the test, the free copper concentration would be initially high and would gradually fall to equilibrium concentration levels throughout the experiment (Figure 15). As the Ma et al. (1999) results indicated, it would take approximately 24 hours for this equilibration to occur. Equilibrium conditions could more closely be matched throughout the test if a 24-hour pre-equilibration period is used (Figure 16). In this test design, the metal is added to the sample containing NOM 24 hours before the organism is introduced. Most of the test duration after this 24-hour period will exhibit equilibrium conditions. Other test designs show even greater sensitivity to these kinetic effects. If a static test is employed where the solution is renewed every 24 hours with a sample aliquot with fresh metal addition, then this period of elevated free copper is repeated with each renewal (Figure 17). Finally, a flowthrough test with a 1-hour HRT should have constant but elevated free copper over then entire test duration compared to the free copper concentrations we would expect at equilibrium (Figure 18). The conditions shown in Figure 18, which are designed to emulate the conditions typical of the Erickson et al. (1996) flowthrough tests with fathead minnow, indicate that copper may easily be 10 times more bioavailable than they would be after a 24-hour equilibration period.

Comparison of BLM predictions to measured LC50 values in the flow-through exposures conducted by Erickson et al. (1987, 1996) show that the BLM over-predicts the measured LC50 values (Figure 19). The amount that the model over-predicts LC50 values is well within this estimate that kinetic

factors may make copper 10 times more bioavailable in flowthrough tests with a 1-hour HRT used in Erickson et al. (1996). Issues that might arise from this equilibrium assumption and its applicability to the use of the BLM in a regulatory context should be addressed. However, it should also be noted that the current water effect ratio procedure does not require that toxicity tests are performed in such a way as to mimic any non-equilibrium conditions at a site.

3.5 Parameter Estimation for Other Organisms

The BLM input parameters that have been evaluated on the basis of the accumulation datasets of Playle and co-workers (1992 and 1993a) and the calibration of the model with the fathead minnow toxicity dataset of Erickson and co-workers (1987 and 1996) serve as the principal basis for model input values for other organisms. This approach is adopted out of necessity, given the absence of accumulation studies for the variety of organisms for which computations would need to be performed. The practical reason for this datagap is that it would be exceedingly difficult to sample the gill or other respiratory tissue in an organism as small as some of the sensitive invertebrates that are commonly used for test purposes (e.g., *D. magna* or *C. dubia*). The possibility that a generally consistent set of biotic ligand parameter values for binding to the biotic ligand would be applicable across organism types is considered plausible, however, if the mechanism of toxicity is the same across all organism types to which the model is applied. This seems to be a reasonable expectation, given that essentially all aquatic organisms must regulate the ionic composition of their internal fluids in order to survive and further, that essentially all forms of aquatic life utilize Na,K-ATPase, the presumed biotic ligand, to do so.

The principal model parameter value that is adjusted for different organisms is the LA50. The reason is that different organisms appear to exhibit varying sensitivities to copper, and increasing or decreasing the LA50 has the effect of increasing or decreasing the predicted dissolved copper concentration that would be in equilibrium with the LA50. The reasons for this varying sensitivity across organism types are not well understood, and this is currently an area of active investigation. One explanation that is related to the known inhibition of active sodium uptake by copper is that the more sensitive organisms have a relatively high turnover rate of exchangeable whole-body sodium levels (Grosell et al. 2002). Thus, if the process of sodium uptake is inhibited, lethal changes in internal sodium pools will occur relatively quickly. Similarly, for a fixed perturbation of internal concentrations of sodium, less inhibition of the sodium uptake is required, such that lower concentrations of copper may be needed to achieve this lesser degree of inhibition. If this explanation is correct, then it would be necessary to evaluate these changes in internal sodium levels to predict toxic effects. Although such models are being developed, they are not currently at the stage of development where they can be routinely applied. As a practical expedient then, the LA50 is adjusted as a way to effectuate this change in sensitivity in the context of a chemical equilibrium-based model framework.

The initial dataset to which the BLM was used to test its applicability to invertebrates was a WER dataset in which *D. pulex* was the test organism. Calibration of the LA50 resulted in a value of 0.035 nmol Cu/g_w used for this analysis. The water chemistry in these model simulations represents conditions in Connecticut streams to which wastewater effluent is discharged (Dunbar, 1996b). In

consideration of the simplified approach that was used to adapt the fathead minnow model to reproduce these data (i.e., it was achieved with the adjustment of a single model parameter, the LA50), the resulting agreement between model and data is encouraging (Figure 20). The use of the LA50 as a means for adjusting the response of the model to more sensitive organisms is attractive from a practical perspective because only the sensitivity of the model is affected. The response of the model to changes in chemistry is determined by log K values and is not affected by changes in the LA50. In the absence of any specific information about the nature of the biotic ligand in *D. pulex*, the adjustment of the LA50 is the simplest means of adapting the fathead minnow parameter set.

This approach seems to also work across numerous datasets. Additional copper toxicity data for *D. pulex* were obtained from Table 1 of the US EPA ambient water quality criteria for copper (J. Mitchell and C. Roberts, personal communication). The same LA50 used for the Dunbar WER study predicts copper toxicity that agrees well with measured LC50s for these data as well (Figure 20). The adjustment of the LA50 value appears to be a simple way to adjust the model to match the varying sensitivity of various organisms to copper toxicity, while avoiding the need to develop independent sets of thermodynamic parameters to different organisms. Additional testing of this approach with data for additional organisms from Table 1 further shows the practical utility of adjusting the LA50. Using this approach, the model was developed for several invertebrates including daphnids (*D. magna*, *D. pulicaria*, *D. pulex*, and *C. dubia*) as well as *H. azteca*. Despite the practical benefits of adjusting the LA50 this way, the low value for the LA50 that results in the *D. pulex* calibration is not likely to be mechanistically meaningful.

4.0 MODEL TESTING: A DESCRIPTION OF MODEL VALIDATION EFFORTS

This section focuses on the testing of the BLM by its application to bioassay datasets obtained with natural water samples, or mixtures of natural waters and Publically Owned Treatment Work (POTW) effluents.

4.1 WER Datasets

The ability of the BLM to reproduce the effect of variations in water chemistry on copper toxicity to fish is, in itself, of interest. However, of more practical significance is its potential use in setting permit limits and defining site-specific water quality criteria (WQC). One component of this procedure is to conduct bioassays to develop water effect ratios (WERs) (USEPA, 1994; Allen and Hansen, 1996). The WER is defined as the ratio of the LC50 in the receiving water to the LC50 in laboratory water for the species being tested. The WQC is then multiplied by the WER to define a site-specific WQC.

4.1.1 Model Testing - Water Effect Ratio Studies Using Fathead Minnow

An example of this procedure is the WER study by Diamond et al. (1997) for copper in a stream in Pennsylvania. The water quality characteristics of the laboratory, upstream, effluent, and mixtures of the effluent and receiving water are summarized in Figure 22. The thermodynamic constants used for WHAM and the gill computations are listed in Tables 1 and 2, respectively. Total organic carbon (TOC) concentrations were approximately 1 mg/L in the lab water, 3 mg/L in the upstream receiving water (U/S), and 12 mg/L in the effluent. Alkalinity (70 to 100 mg CaCO₃/L) and hardness (75 to 160 mg CaCO₃/L) varied to a lesser degree, while the pH was relatively constant in these waters (pH ~ 8). The water quality characteristics of the mixtures of upstream and effluent water were generally consistent with what would be predicted from mass balances, with the exception of the 75% effluent sample for alkalinity and hardness.

The procedure used to predict the WER for fathead minnows is as described above. That is, the water quality characteristics for a test sample are used in the BLM to compute the gill copper concentration. As with the other natural water datasets that have been analyzed with the model, dissolved organic carbon (DOC) inputs to the BLM was assumed to be 10% humic acid and 90% fulvic acid for these waters. A numerical titration is performed in which the dissolved copper is varied over a range of concentrations, and the computed gill copper concentration is determined for each concentration of copper added. Representative results are illustrated for an effluent sample and a laboratory sample in Figure 13. For the effluent sample the gill copper concentration initially increases hardly at all as copper is added to the system, reflecting the complexation of the copper by DOC high-affinity binding sites. Once the high-affinity binding sites are saturated, the gill copper concentration begins to increase more rapidly as copper is added. The dissolved copper concentration corresponding to a gill copper concentration of 6 nmol/g_w, the gill copper LC50, is 1450 µg/L. This dissolved copper concentration is used as the predicted LC50 for copper in the effluent.

In contrast, the predicted gill copper concentration in laboratory water increases much more rapidly as copper is added. The more rapid increase occurs because of the lower concentration of DOC in laboratory water. Hence, less Cu-DOC complex forms. This, together with lower alkalinity that produces a lower concentration of copper carbonate complexes, and lower hardness so that there is less calcium competition, results in a faster rate of increase in gill copper concentration as dissolved copper concentration increases. The result is that the predicted gill copper concentration reaches the gill Cu LC50 of 6 nmol/g_w at a much lower dissolved copper concentration of about 200 µg/L. This concentration is the predicted LC50 for copper in the laboratory water. The ratio of the predicted effluent to laboratory water LC50s, $1,450/200 = 7.25$, is the predicted WER.

A summary of the observed and predicted results of the WER analysis for the January test data is shown in Figure 23. The dissolved copper LC50s for the lab water, upstream water, mixtures of upstream and effluent (53 and 75 percent effluent), and 100 percent effluent are compared in Figure 23 (upper panel). The general trend of increasing LC50 with increasing effluent is reproduced for both the observed results and model predictions, with the exception of the 75% effluent sample for which the measurements of alkalinity and hardness are inconsistent with the mixing of the end-members (Figure 22). Analogous results for the predicted WERs are also shown in Figure 23 (lower panel). Since both the predicted and measured lab water LC50s are used to compute the WER, the lab water WER equals one (by definition). The predicted WER values for the remaining samples

agree well with measured values, showing the utility of BLM results for calculating a site-specific water quality criteria in an approach that is analogous to the WER.

Additional test datasets were obtained from WER studies using fathead minnow to determine site-specific water quality criteria for copper in effluent impacted streams in Connecticut (Dunbar et al., 1996b). Since it is not yet known how organic matter in municipal waste streams compares to that from natural sources, it is assumed here that the DOC can be described using the same distribution of 10% humic and 90% fulvic acids. Given these assumptions, application of the BLM to the Connecticut dataset yields good agreement between predicted and measured values (Figure 24). In summary, considering both the Erickson et al. (1996) laboratory test data and the Pennsylvania and Connecticut WER data, nearly all of the predicted results are within a factor of two of the measured values.

Another WER data from Pennsylvania rivers used to test the BLM was developed by Hall et al. (1998) for the Pennsylvania Copper Group. These data included a group of 10 municipal wastewater treatment plants that discharge to ten streams in Pennsylvania. The *C. dubia* bioassays were run with laboratory water and for two different dilutions of wastewater with receiving water. As a result, there was considerable variation in copper toxicity. The BLM predictions for these same conditions compare favorably with these measurements as well as for other *C. dubia* data from Table 1 of the copper criteria document (Figure 25), showing the ability of the BLM to account for differences in copper toxicity over a wide range of conditions.

5.0 MODEL APPLICATION

The BLM is applicable to a variety of water and organism types. However, there are limits with regard to the applicability at its current state of development. This section describes some of the conditions over which the model has been applied, and limitations of BLM applicability as well.

5.1 Applicability and Limitations

5.1.1 Equilibrium Assumptions

To date the BLM has primarily been applied in the analysis of toxicity results obtained under laboratory test conditions, with both synthetic waters and natural waters. It is assumed that equilibrium conditions prevail amongst the various chemical components in the test water, including the biotic ligand, and the model has been calibrated under this assumption. Results presented in Section 4 highlighted the problem that can be encountered when this is not the case. As stated above, issues that might arise from this equilibrium assumption and its applicability to the use of the BLM in a regulatory context should be addressed. However, it should also be noted that the current water effect ratio procedure does not require that toxicity tests are performed in such a way as to mimic any non-equilibrium conditions at a site. Thus, under the current state of development of the BLM, this limitation should not be viewed as a disadvantage of the BLM relative to other methods

that are currently available to set WQC.

5.1.2 Aquatic Organisms

To date the BLM for copper has been calibrated with acute toxicity datasets for the following aquatic organisms:

Freshwater: fathead minnow (*P. promelas*), rainbow trout, *Daphnia magna*, *D. pulex*, *D. pulicaria*, *Hyallela azteca*, *Ceriodaphnia dubia*.

Saltwater: *Mytilus edulis*

The degree of calibration varies by organism, as does the range of water quality conditions tested.

Preliminary analyses have been performed with USEPA WQC Table 1 data in instances where sufficient water chemistry was available or could be reasonably estimated (USEPA, 2003a). While these analyses are not considered definitive, on balance it appears that the BLM reduces the degree of uncertainty in the results, with regard to its ability to predict LC50 levels, in comparison to a more simplified hardness-based analysis. This is so in spite of the fact that the preponderance of the data in the WQC document tend to reflect hardness variation, in comparison to other test conditions, such as pH, alkalinity and DOC. As a result, it is concluded that the BLM for copper provides an improved assessment tool in comparison to the hardness-based approach.

Other studies are ongoing to develop bioassay datasets that can be used to refine the current parameterization of the BLM. Such results will be able to be readily incorporated into updated releases of the BLM, over time, as appropriate.

5.1.3 Ranges of Input Parameters Used in Calibration

Input parameters upon which the BLM calibration is based are summarized previously in Section 3.0. This summary does not reflect any multi-variable interactions that are present in the database. Further information on the applicable range of water quality characteristics and organism types is presented in the BLM Users Guide (USEPA, 2003b).

5.1.4 Other Considerations

Only a limited effort has been put forth in the application of the BLM to saltwater datasets to date (Di Toro et al., 2000). Thus, the Version 2.2.0 of the BLM is not currently viewed as being suitable for use in the evaluation of saltwater WQC. As a result, standard hardness-based WQC methods will be applied to evaluate saltwater WQC in the immediate future.

To date the model is only considered to be appropriate for use in the evaluation of acute toxicity. Although future work is planned to extend the applicability of the model to chronic toxicity, it is not

recommended that Version 2.2.0 of the BLM be used for chronic toxicity evaluations at this time. Thus, existing procedures, involving application of an acute to chronic ratio (ACR), will be used until a BLM-based chronic toxicity model has been developed and judged to be acceptable for use.

6.0 REFERENCES

Allen, H. E. and D. J. Hansen, January 1996. "The Importance of Trace Metal Speciation to Water Quality Criteria," *Water Environment Research*, 68(1): 42-54.

Allison, J.D., D.S. Brown, K.J. Novo-Gradac, March 1991. "MINTEQA2/PRODEFA2, A Geochemical Assessment Model for Environmental Systems: Version 3.0, Users Manual," EPA/600/3-91/021, USEPA ERL ORD, Athens, GA.

Ankley, G.T., D.M. Di Toro, D.J. Hansen, W.J. Berry, December, 1996. "Technical Basis and Proposal for Deriving Sediment Quality Criteria for Metals," *Environmental Toxicology and Chemistry*, 15: 2056-2066.

Bell, R. and J. Kramer, 1999. "Structural Chemistry and Geochemistry of Silver-Sulfur Compounds: Critical Review," *Environmental Toxicology and Chemistry*, 19: 9-22.

Benedetti, M.F., C.J. Milne, D.G. Kinniburgh, W.H. Van Riemsdijk and L.K. Koopal, 1995. "Metal Ion Binding to Humic Substances: Application of the Non-Ideal Competitive Adsorption Model," *Environmental Science and Technology*, 29: 446-457.

Bergman, H.L., and E.J. Dorward-King, eds., 1997. *Reassessment of Metals Criteria for Aquatic Life Protection: Priorities for Research and Implementation*, Proceedings of the SETAC Pellston Workshop on Reassessment of Metals Criteria for Aquatic Life Protection, February 10-14, 1996, Pensacola, Florida, SETAC Press, 114 pp.

Bills, T., S.J. Klaine, T.W. LaPoint, G. Cobb, B. Forsythe and M. Wenholz, June 1997. "Influence of Water Quality Parameters on Acute Silver Nitrate Toxicity to *Daphnia magna*," Final Report No. TIWET-9506.1, submitted to the Silver Council c/o the National Association of Photographic Manufacturers by the Department of Environmental Toxicology, The Institute of Wildlife and Environmental Toxicology (TIWET), Clemson University, Pendleton, SC.

Brown, D.S. and J.D. Allison, February, 1987. "MINTEQA1 Equilibrium Metal Speciation Model: A User's Manual," prepared for the USEPA Environmental Research Laboratory, Office of Research and Development, Athens, Georgia.

Brown, P.L. and S.J. Markich, 2000. Evaluation of the free ion activity model of metal-organism interaction: extension of the conceptual model. *Aquatic Toxicology*, 51(2): 177-194

Bury, N.R., F. Galvez and C.M. Wood, 1999. "Effects of Chloride, Calcium and Dissolved Organic

Carbon on Silver Toxicity: Comparison Between Rainbow Trout and Fathead Minnows,” *Environmental Toxicology and Chemistry*, 18(1) 56-62.

Campbell, PGC, 1995. “Interactions Between Trace Metals and Aquatic Organisms: A Critique of the Free-ion Activity Model,” in *Metal Speciation and Bioavailability in Aquatic Systems*, A. Tessier and D.R. Turner, eds., John Wiley and Sons, New York, pp. 45-102.

Chakoumakos, C., R.C. Russo and R.V. Thurston. 1979. The toxicity of copper to cutthroat trout (*Salmo clarki*) under different conditions of alkalinity, pH, and hardness. *Environ. Sci. Technol.* 13:213-219.

Diamond, J.M., C. Gerardi, E. Leppo and T. Miorelli, 1997. “Using a Water-effect Ratio Approach to Establish Effects of an Effluent-influenced Stream on Copper Toxicity to the Fathead Minnow,” *Environmental Toxicology and Chemistry*, 16: 1480-1487.

Di Toro, D.M., J.A. Hallden and J.L. Plafkin, 1988. “Modeling *Ceriodaphnia* Toxicity in the Naugatuck River Using Additivity and Independent Action,” in *Toxic Contaminants and Ecosystem Health: A Great Lakes Focus*, John Wiley, New York, NY, USA, pp. 403-425.

Di Toro, D.M., J.A. Hallden and J.L. Plafkin, 1991. “Modeling *Ceriodaphnia* Toxicity in the Naugatuck River. 2. Copper, Hardness and Effluent Interactions,” *Environmental Toxicology and Chemistry*, 10: 261-274.

Di Toro, D.M., C.S. Zarba, D.J. Hansen, W.J. Berry, R.C. Schwartz, C.E. Cowan, S.P. Pavlou, H.E. Allen, N.A. Thomas and P.R. Paquin, 1991. “Technical Basis for Establishing Sediment Quality Criteria for Nonionic Organic Chemicals by Using Equilibrium Partitioning,” *Environmental Toxicology and Chemistry*, 10(12): 1541-1583.

Di Toro, D.M. and J.A. McGrath, January 2000. “Technical Basis for Narcotic Chemicals and Polycyclic Aromatic Hydrocarbon Criteria. 2. Mixtures and Sediments,” *Environmental Toxicology and Chemistry*, 19: 1971-1982.

Di Toro, D.M., H.E. Allen, H.L. Bergman, J.S. Meyer, P.R. Paquin and R.C. Santore, August 2000. “The Biotic Ligand Model: A Computational Approach for Assessing the Ecological Effects of Metals in Aquatic Systems,” published by the International Copper Association, Ltd., Environmental Program, as part of its series on “*Copper in the Environment and Health*”.

Di Toro, D.M., H.E. Allen, H.L. Bergman, J.S. Meyer, P.R. Paquin and R.C. Santore, 2001. “A Biotic Ligand Model of the Acute Toxicity of Metals. I. Technical Basis,” *Environmental Toxicology and Chemistry*. 20:2383-2396.

Dunbar, L.E., February 1996a. “Effect of Streamflow on the Ability of Ambient Waters to Assimilate Acute Copper Toxicity,” a paper presented at the 20th Meeting of the New England Association of Environmental Biologists, Falmouth MA, Connecticut DEP, Water Toxics Program.

Dunbar, L.E., 1996b. "Derivation of a Site-Specific Dissolved Copper Criteria for Selected Freshwater Streams in Connecticut," Falmouth MA, Connecticut DEP, Water Toxics Program, Falmouth, MA. (An excerpt from an uncited report that was received from author)

Dzombak, D., and F. Morel, 1990. *Surface Complexation Modeling. Hydrous Ferric Oxide*, John Wiley, New York, NY, USA.

Erickson, R.J., D.A. Benoit and V.R. Mattson, 1987. "A Prototype Toxicity Factors Model For Site-Specific Copper Water Quality Criteria," revised September 5, 1996, United States Environmental Protection Agency, Environmental Research Laboratory-Duluth, Duluth, MN.

Erickson, R.J., D.A. Benoit, V.R. Mattson, H.P. Nelson Jr. and E.N. Leonard, 1996. "The Effects of Water Chemistry on the Toxicity of Copper to Fathead Minnows," *Environmental Toxicology and Chemistry*, 15(2): 181-193.

Grosell, M., C. Nielsen and A. Bianchini. 2002. Sodium turnover rate determines sensitivity to acute copper and silver exposure in freshwater animals. *Comp. Biochem. Physiol. Part C* 133:287-303.

Hall and Associates and Environmental Engineering and Management Associates, February 26, 1998. "Evaluation of Copper Toxicity and Water Effect Ratio for Treated Municipal Wastewater," Final Report prepared for the Pennsylvania Copper Group.

Hollis, L., K. Burnison and R.C. Playle, 1996. "Does the Age of Metal-Dissolved Organic Carbon Complexes Influence Binding of Metals to Fish Gills?," *Aquatic Toxicology*, 35: 253-264.

Hollis, L., L. Muench and R.C. Playle, 1997. "Influence of Dissolved Organic Matter on Copper Binding, and Calcium on Cadmium Binding, by Gills of Rainbow Trout," *Journal of Fish Biology*, 50: 703-720.

Janes, N., and R.C. Playle, 1995. "Modeling Silver Binding to Gills of Rainbow Trout (*Oncorhynchus mykiss*)," *Environmental Toxicology and Chemistry*, Vol. 14, No. 11, 1847-1858.

Kinniburgh, D.G., C.J. Milne, M.F. Benedetti, J.P. Pinheiro, J. Filius, L.K. Koopal, and W.H. Van Riemsdijk, 1996. "Metal Ion Binding by Humic Acid: Application of the NICA-Donnan Model," *Environmental Science and Technology*, 30: 1687-1698.

Ma, H., S.D. Kim, D.K. Cha and H.E. Allen, 1999. "Effect of Kinetics of Complexation by Humic Acid on Toxicity of Copper to *Ceriodaphnia dubia*," *Environmental Toxicology and Chemistry*, 18: 828-837

MacRae, R.K., December, 1994. "The Copper Binding Affinity of Rainbow Trout (*Oncorhynchus mykiss*) and Brook Trout (*Salvelinus fontinalis*) Gills," a thesis submitted to the

Department of Zoology and Physiology and The Graduate School of the University of Wyoming in partial fulfillment of the requirements for the degree of Master of Science in Zoology and Physiology.

MacRae, R.K., D.E. Smith, N. Swoboda-Colberg, J.S. Meyer and H.L. Bergman, 1999. "Copper Binding Affinity of Rainbow Trout (*Oncorhynchus mykiss*) and Brook Trout (*Salvelinus fontinalis*) Gills," *Environmental Toxicology and Chemistry*, 18: 1180-1189.

McDonald, D.G., J.P. Reader and T.R.K. Dalziel, 1989. "The Combined Effects of pH and Trace Metals on Fish Ionoregulation," in: *Acid Toxicity and Aquatic Animals*, edited by R. Morris, E.W. Taylor, D.J.A. Brown and J.A. Brown, Soc. Exp. Biol. Seminar Series, Vol. 34, Cambridge University Press, Cambridge, U.K., pp. 221-242.

Meyer, J.S., 1999a. "A Mechanistic Explanation for the $\ln(\text{LC50})$ vs. $\ln(\text{Hardness})$ Adjustment Equation for Metals," *Environ. Sci. Technol.*, 33: 908-912.

Meyer, J.S., R.C. Santore, J.P. Bobbitt, L.D. DeBrey, C.J. Boese, P.R. Paquin, H.E. Allen, H.L. Bergman and D.M. DiToro, 1999b. "Binding of Nickel and Copper to Fish Gills Predicts Toxicity When Water Hardness Varies, But Free-ion Activity Does Not," *Environmental Science and Technology*, 33: 913-916.

Meyer, J.S., R.C. Santore, J.P. Bobbitt, L.D. DeBrey, C.J. Boese, P.R. Paquin, H.E. Allen, H.L. Bergman and D.M. Di Toro. 1999. Binding of nickel and copper to fish gills predicts toxicity when water hardness varies, but free-ion activity does not. *Environ. Sci. Technol.* 33:913-916.

Meyer, J.S. 2002a. The utility of the terms "bioavailability" and "bioavailable fraction" for metals. *Mar. Environ. Res.* 53:417-423.

Meyer, J.S., C.J. Boese and S.A. Collyard. 2002b. Whole-body accumulation of copper predicts acute toxicity to an aquatic oligochaete (*Lumbriculus variegatus*) as pH and calcium are varied. *Comp. Biochem. Physiol. Part C* 133:99-109.

Morel, F.M., 1983. "Complexation: Trace Metals and Microorganisms," in Chapter 6 of *Principles of Aquatic Chemistry*, Wiley Interscience, New York, pp. 301-308.

Morel, F.M.M. and J.G. Hering, 1993. *Principles and Applications of Aquatic Chemistry*, 2nd Edition, a Wiley Interscience Publication, John Wiley and Sons, New York.

Pagenkopf, G.K., R.C. Russo and R.V. Thurston, 1974. "Effect of Complexation on Toxicity of Copper to Fishes," *Journal of Fisheries Research Board of Canada*, 31: 462-465.

Pagenkopf, G.K., 1983. "Gill Surface Interaction Model for Trace-metal Toxicity to Fishes: Role of Complexation, pH, and Water Hardness," *Environmental Science and Technology*, 17: 342-347.

Paquin, P.R., D.M. Di Toro, R.C. Santore, D. Trivedi and K.B. Wu, April 1999. "A Biotic Ligand Model of the Acute Toxicity of Metals. III. Application to Fish and *Daphnia* Exposure to Silver," Section 3 in *Integrated Approach to Assessing the Bioavailability and Toxicity of Metals in Surface Waters and Sediments*, a submission to the EPA Science Advisory Board, Office of Water, Office of Research and Development, Washington, DC, pp. 3-59 to 3-102. USEPA-822-E-99-001.

Playle, R.C., R.W. Gensemer and D.G. Dixon, 1992. "Copper Accumulation on Gills of Fathead Minnows: Influence of Water Hardness, Complexation and pH on the Gill Micro-environment," *Environmental Toxicology and Chemistry*, 11: 381-391.

Playle, R.C., D.G. Dixon and K. Burnison, 1993a. "Copper and Cadmium Binding to Fish Gills: Modification by Dissolved Organic Carbon and Synthetic Ligands," *Can. J. Fish. Aquat. Sci.*, 50: 2667-2677.

Playle, R.C., D.G. Dixon and K. Burnison, 1993b. "Copper and Cadmium Binding to Fish Gills: Estimates of Metal-Gill Stability Constants and Modeling of Metal Accumulation," *Can. J. Fish. Aquat. Sci.*, 50: 2678-2687.

Playle, R.C., 1998. "Modelling Metal Interactions at Fish Gills," *The Science of the Total Environment*, 219: 147-163.

Renner, R., 1997. "Rethinking Water Quality Standards for Metals Toxicity," *Environmental Science and Technology*, 31: 466-468.

Richards, J. and R. Playle, 1998. "Cobalt Binding to Gills of Rainbow Trout (*Oncorhynchus mykiss*): An Equilibrium Model," *Comp. Biochem. Physiol. C*, 119:185-197.

Santore, R.C. and C.T. Driscoll, 1995. "The CHESS Model for Calculating Chemical Equilibria in Soils and Solutions," *Chemical Equilibrium and Reaction Models*, R. Loeppert, A.P. Schwab and S. Goldberg, eds., SSSA Special Publication 42, The Soil Society of America, American Society of Agronomy, Madison, WI, pp. 357-375.

Santore, R.C., D.M. Di Toro and P.R. Paquin, H.E. Allen, and J.S. Meyer. 2001. "A Biotic Ligand Model of the Acute Toxicity of Metals. II. Application to Acute Copper Toxicity in Freshwater Fish and *Daphnia*," *Environmental Toxicology and Chemistry*. 20:2397-2402.

Smith, R. and R. Martell, 1993. *NIST Critical Stability Constants of Metal Complexes Database, NIST Standard Reference Database 46*, National Institute of Standards and Technology, Gaithersburg, MD, USA.

Sprague, J.B. and B.A. Ramsay, 1965. "Lethal Levels of Mixed Copper-Zinc Solutions for Juvenile Salmon," *Journal of Fisheries Research Board Canada*, 22: 425-432.

Tipping, E., C.A. Backes and M.A. Hurley, 1989. "Modeling the Interactions of Al Species, Protons, and ca with Humic Substances in Acid Waters and Soils," in *Environmental Chemistry and Toxicology of Aluminum*, T.E. Lewis, ed., Lewis, London, pp. 59-82.

Tipping, E., and M. Hurley, 1992. "A Unifying Model of Cation Binding by Humic Substances," *Geochim Cosmochim Acta*, 56: 3627-3641.

Tipping, E., 1993. "Modeling the Competition Between Alkaline Earth Cations and Trace Metal Species for Binding by Humic Substances," *Environmental Science and Technology*, 27: 520-529.

Tipping, E., 1994. "WHAM--A Chemical Equilibrium Model and Computer Code for Waters, Sediments, and Soils Incorporating a Discrete Site/Electrostatic Model of Ion-Binding by Humic Substances," *Computers and Geosciences*, 20(6): 973-1023.

USEPA, January 1985. "Ambient Water Quality Criteria for Copper - 1984," Office of Water Regulations and Standards, Criteria and Standards Division, Washington, DC.

USEPA, August 1994. "Water Quality Standards Handbook: Second Edition, Appendix L, Interim Guidance on Determination and Use of Water Effect Ratios for Metals," USEPA, Office of Water, EPA-823-B-94-005a.

USEPA, April 6-7, 1999. *Integrated Approach to Assessing the Bioavailability and Toxicity of Metals in Surface Waters and Sediments*, a report to the EPA Science Advisory Board, Office of Water, Office of Research and Development, Washington, DC, USEPA-822-E-99-001.

USEPA, February 2000. "An SAB Report: Review of the Biotic Ligand Model of the Acute Toxicity of Metals," prepared by the Ecological Processes and Effects Committee of the Science Advisory Board, EPA-SAB-EPEC-00-0006.

USEPA. 2003a. *Estimation of Water Chemistry Parameters for Acute Copper Toxicity Tests*. Office of Water, Office of Science and Technology, Health and Ecological Criteria Division, Washington, D.C.

USEPA. 2003b. Update of Ambient Water Quality Criteria for Copper. Office of Water, Office of Science and Technology, Health and Ecological Criteria Division, Washington, D.C.

Welsh, P.G., J.F. Skidmore, D.J. Spry, D.G. Dixon, P.V. Hodson, N.J. Hutchinson and B.E. Hickie, 1993. "Effect of Ph and Dissolved Organic Carbon on the Toxicity of Copper to Larval Fathead Minnow (*Pimephales promelas*) in Natural Waters of Low Alkalinity, *Canadian Journal of Fisheries and Aquatic Sciences*, 50: 1356-1362.

Welsh, P.G., J. Lipton, G.A. Chapman and T.L. Podrabsky, 2000. "Relative Importance of Calcium and Magnesium in Hardness-based Modification of Copper Toxicity," *Environmental*

Toxicology and Chemistry, 19: 1624-1631.

Westall, J.C., J.L. Zachary and F. Morel, 1976. "MINEQL, A Computer Program for the Calculation of Chemical Equilibrium Composition of Aqueous Systems," Technical Note 18. Department of Civil Engr. MIT. Cambridge MA.

Westall, J., and H. Hohl, 1980. "A Comparison of Electrostatic Models for the Oxide/Solution Interface," *Advances in Colloid and Interface Science*, 12: 265-294.

Wood, C.M., R.C. Playle and C. Hogstrand, 1999. "Physiology and Modeling of the Mechanisms of Silver Uptake and Toxicity in Fish," *Environmental Toxicology and Chemistry*, 18(1): 71-83.

Table 1. Inorganic copper and organic speciation reactions in the WHAM database (Tipping, 1994).

Organic Complexes	Proton Exchange Constant, pK
Cu-Humic	1.5
Cu-Fulvic	0.8
CuOH-Humic	1.5
CuOH-Fulvic	0.8
Ca-Humic	3.2
Ca-Fulvic	2.2
Mg-Humic	3.3
Mg-Fulvic	2.2
<hr/>	
Inorganic Species Formation Reactions	Log K
$\text{Cu} + \text{OH} = \text{Cu}(\text{OH})$	6.48
$\text{Cu} + 2*\text{OH} = \text{Cu}(\text{OH})_2$	11.78
$\text{Cu} + \text{SO}_4 = \text{CuSO}_4$	2.36
$\text{Cu} + \text{CO}_3 = \text{CuCO}_3$	6.55
$\text{Cu} + 2*\text{CO}_3 = \text{Cu}(\text{CO}_3)_2$	9.92
$\text{Cu} + \text{Cl} = \text{CuCl}$	0.4
$\text{Cu} + \text{H} + \text{CO}_3 = \text{CuHCO}_3$	14.32

Table 2 Stoichiometry and Thermodynamic Constants for Adsorption of Metals and Protons on Gills of Larval Fathead Minnows.

Reaction	Log K
H +gill = H-gill	5.4
Na +gill = Na-gill	3.0
Ca +gill = Ca-gill	3.6
Mg +gill = Mg-gill	3.6
Cu + gill = Cu-gill	7.4

Table 3. Average composition of waters used in the fathead minnow copper toxicity experiments of Erickson et al. (1987) in exposures without chemical modifications.

Water source	DOC mg/L	pH	Ca mg/L	Mg mg/L	Na mg/L	K Mg/L	Cl mg/L	SO4 mg/L	Alkalinity mg CaCO3/L
Lake Superior Water	1.0	7.9	13.5	2.9	1.6	0.4	1.4	6.7	42.5
Synthetic Water	0.1	8.0	91.5	30.3	59.5	5.0	61.7	168.8	146.7

Note: Concentrations are measured, except for DOC concentrations which were provided by the lead author (Erickson, 2000, Personal Communication).

BIOTIC LIGAND MODEL

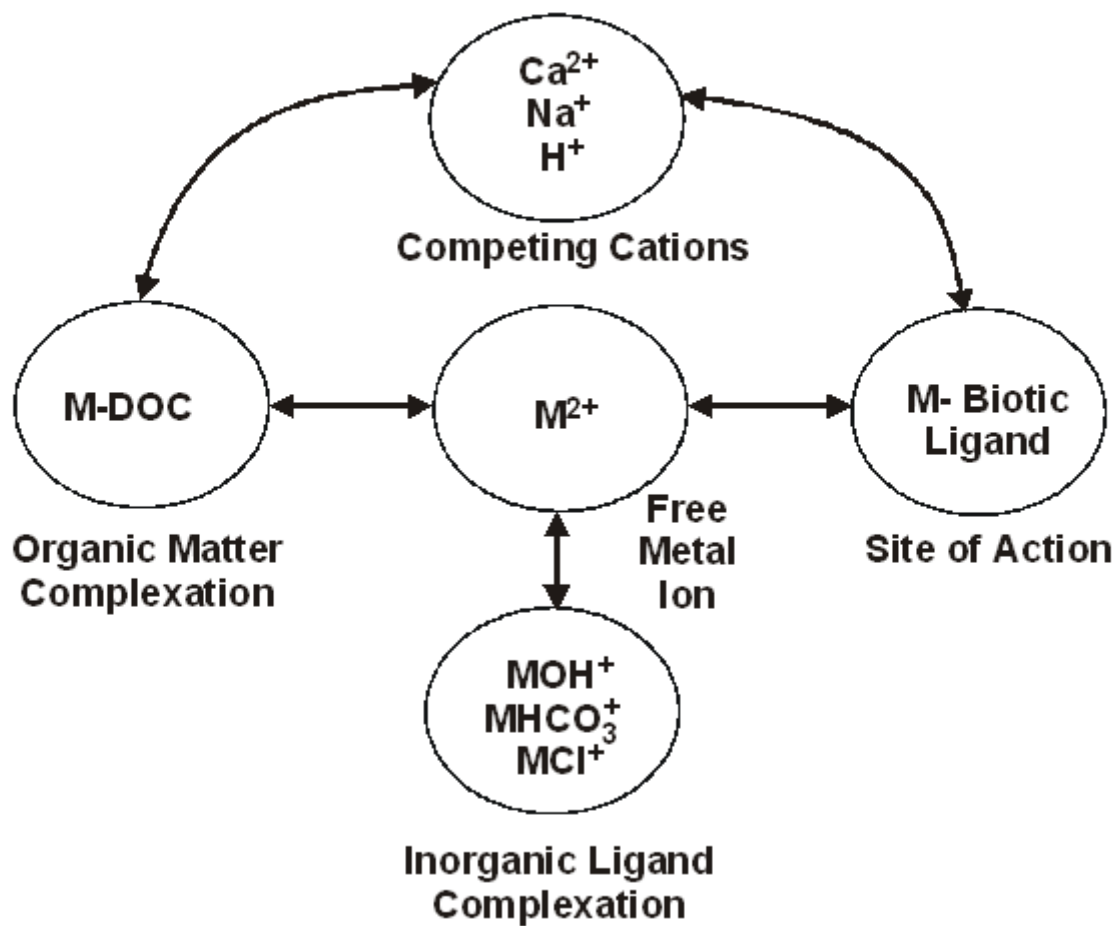


Figure 1 Schematic diagram of the generalized biotic ligand model (BLM) framework for acute toxicity of a divalent cationic metal.

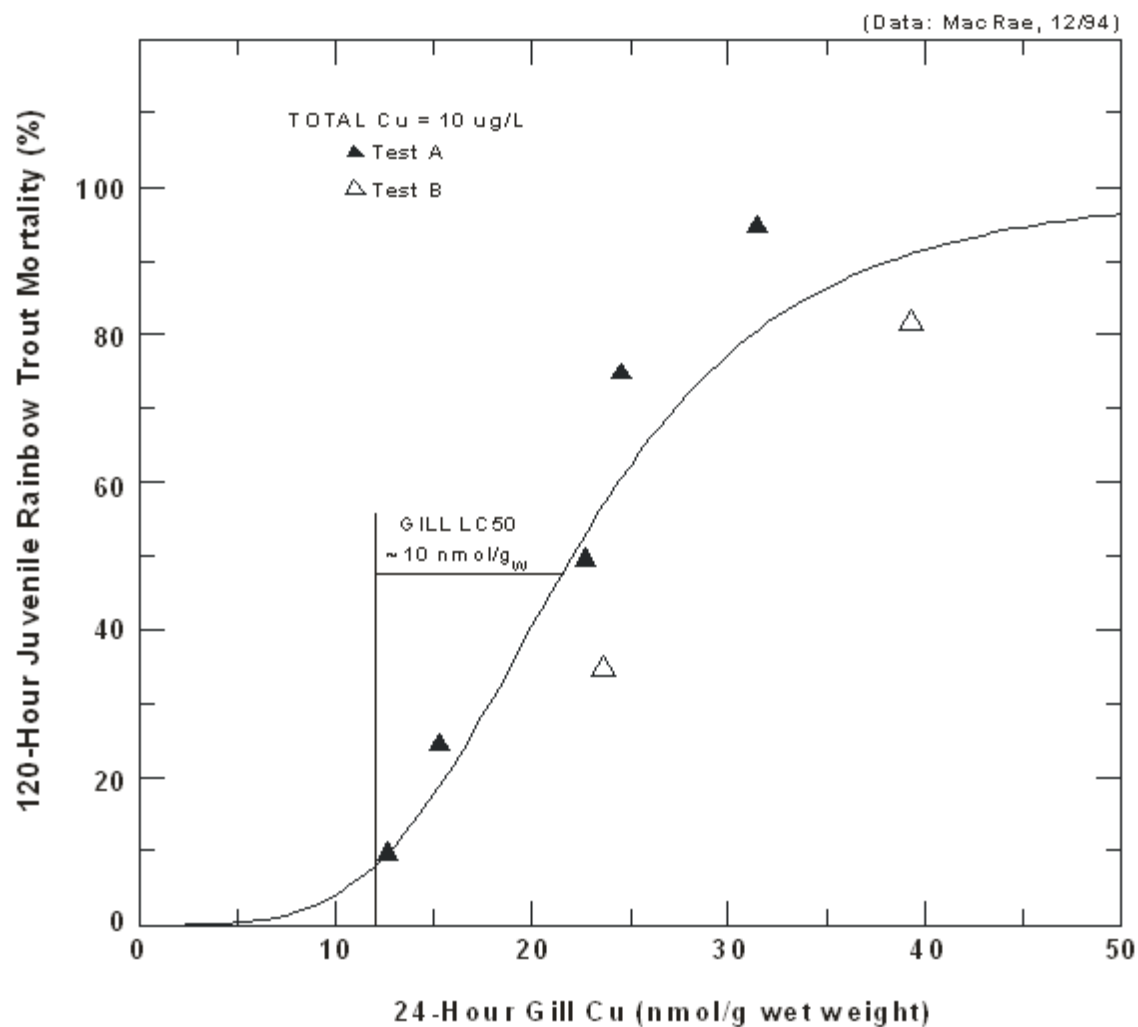


Figure 2 Relationship between mortality of juvenile rainbow trout after 120 hours of exposure and copper concentration on the gill of the fish after 24 hours of exposure. Data from MacRae et al. (1999).

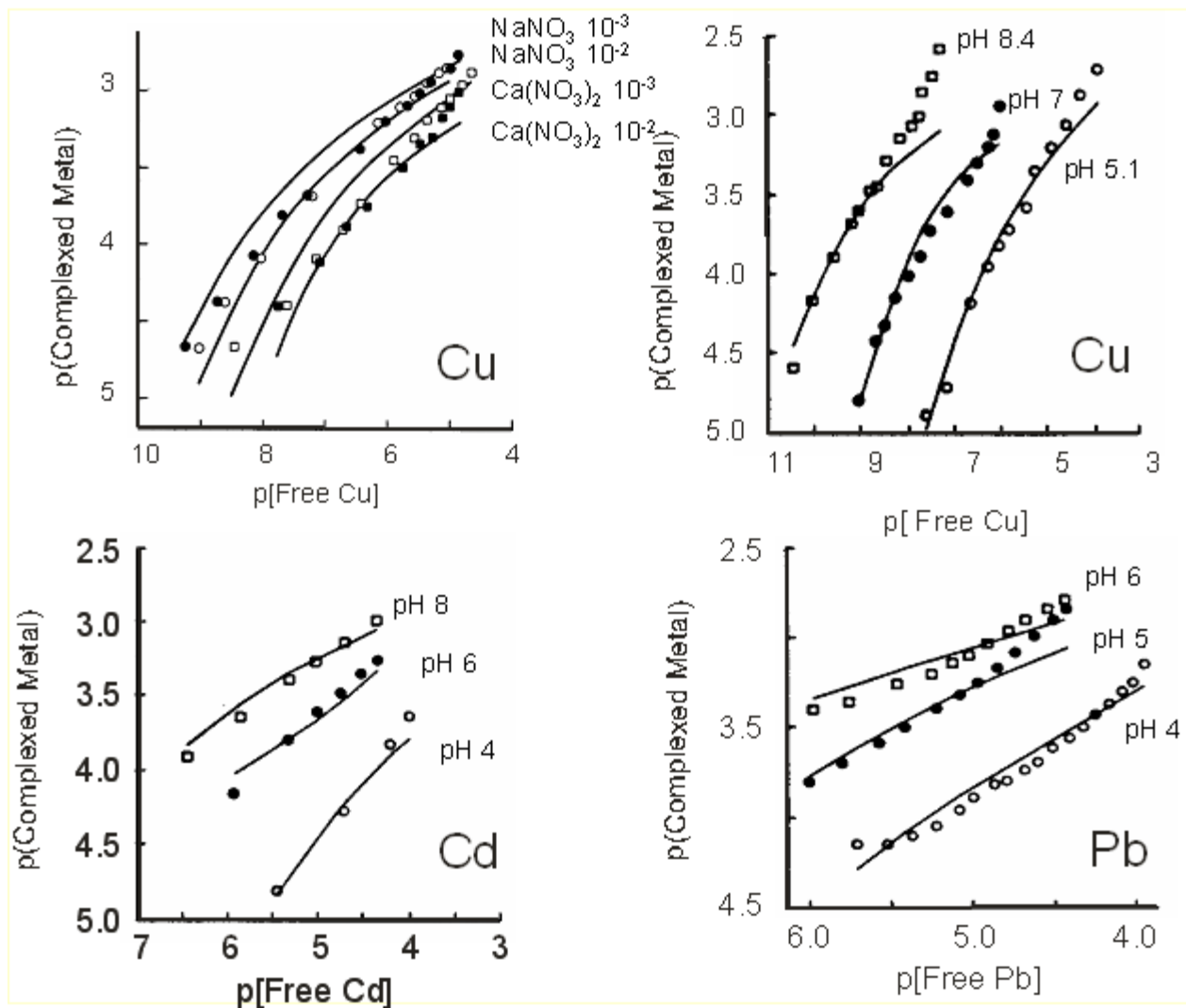


Figure 3 Calibration of the WHAM model, Version V. (A) Cu: effect of ionic strength: 0.001 M (\circ) 0.01 M (\bullet) (B) Cu: effect of Ca: 0.001 M NaNO_3 (\circ) 0.001 M $\text{Ca}(\text{NO}_3)_2$ (\bullet) 0.01 M $\text{Ca}(\text{NO}_3)_2$ (\square) (C) Cu: effect of pH: 5.14 (\circ) 7.00 (\bullet) 8.44 (\square) (D) Ca: effect of pH: 5.00 (\circ) 7.00 (\bullet) 9.00 (\square) (E) Cd: effect of pH: 4.00 (\circ) 6.00 (\bullet) 8.00 (\square) (F) Pb: effect of pH: 4.00 (\circ) 5.00 (\bullet) 6.00 (\square). Redrawn from Tipping and Hurley (1992).

CONCEPTUAL DIAGRAM OF COPPER SPECIATION
AND COPPER-GILL MODEL (After Pagenkopf, 1983)

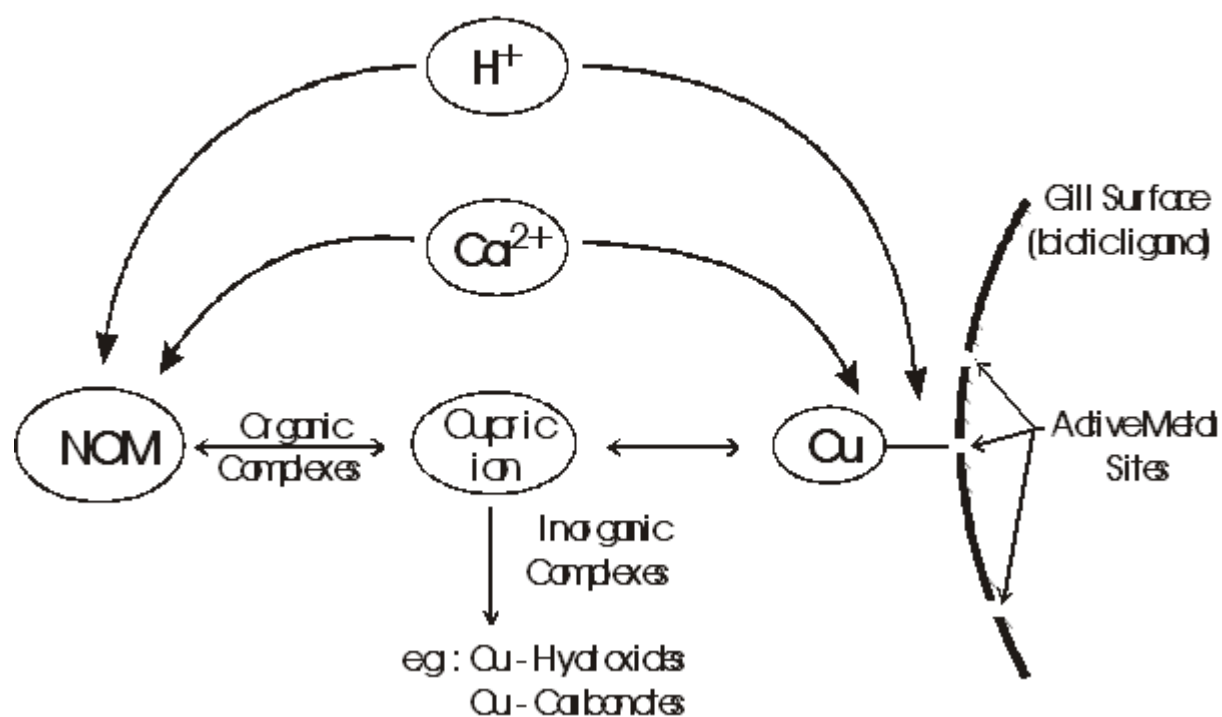


Figure 4 Schematic diagram of the biotic ligand model (BLM) framework for acute copper toxicity, showing inorganic and organic complexation in the water and interaction of metals and cations on the biotic ligand.

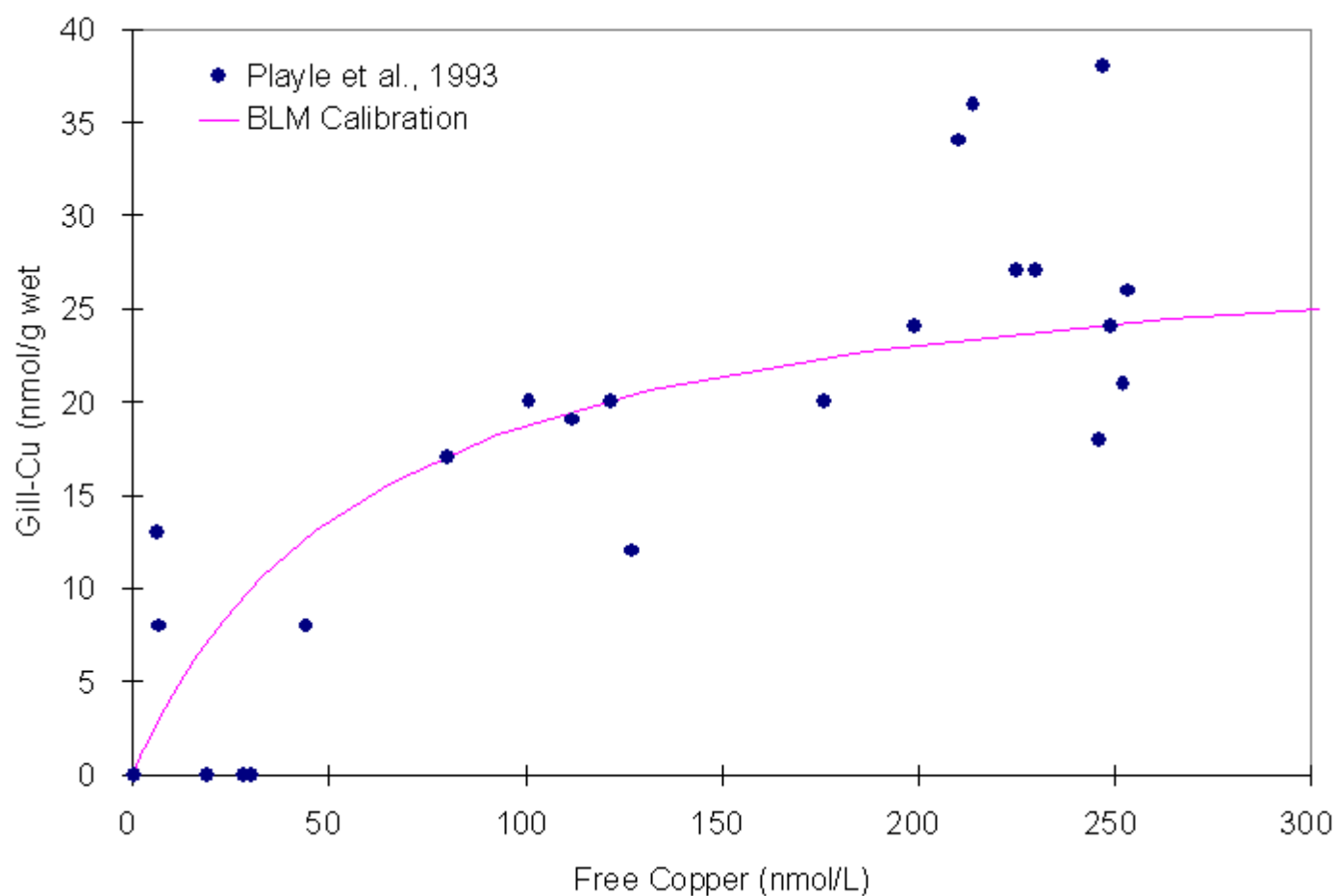


Figure 5 Measured copper accumulation on fathead minnow gills from Playle et al. 1993b; and Biotic Ligand Model predictions as a function of cupric ion concentration.

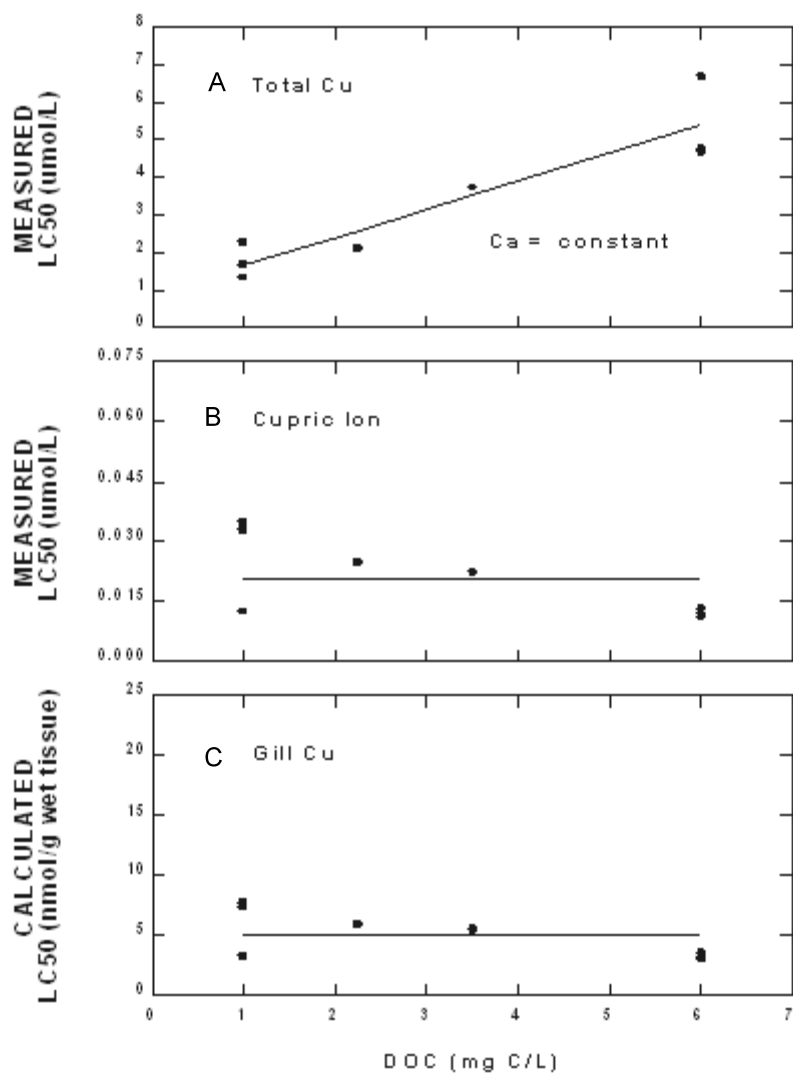


Figure 6 Relationship of copper LC50s to variations in DOC concentration. The lines are drawn by eye to represent the data. (A) LC50 expressed as the concentration of total dissolved copper. (B) LA50 expressed as the concentration of the free ion activity of copper. (C) LC50 expressed as the concentration of the copper sorbed to the gill. Data from Erickson et al. (1996).

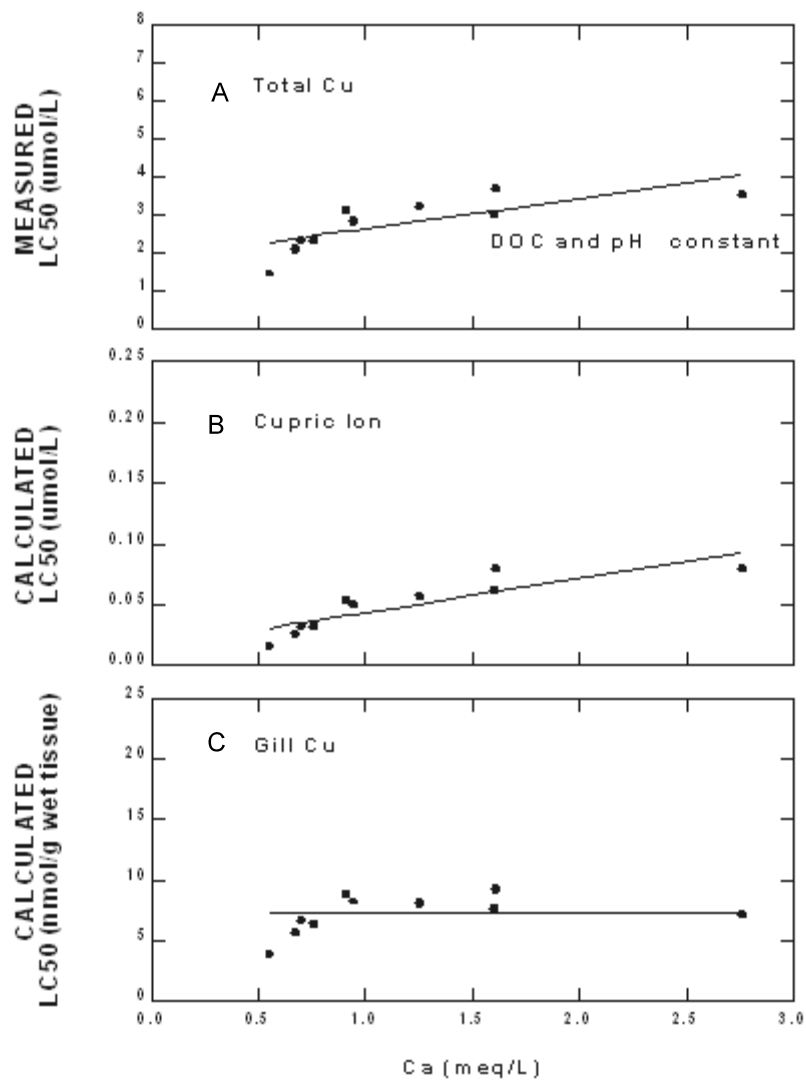


Figure 7 Relationship of copper LC50s to variations in calcium concentration. The lines are drawn by eye to represent the data. (A) LC50 expressed as the concentration of total dissolved copper. (B) LC50 expressed as the concentration of the free ion activity of copper. (C) LA50 expressed as the concentration of the copper sorbed to the gill. Data from Erickson et al. (1996).

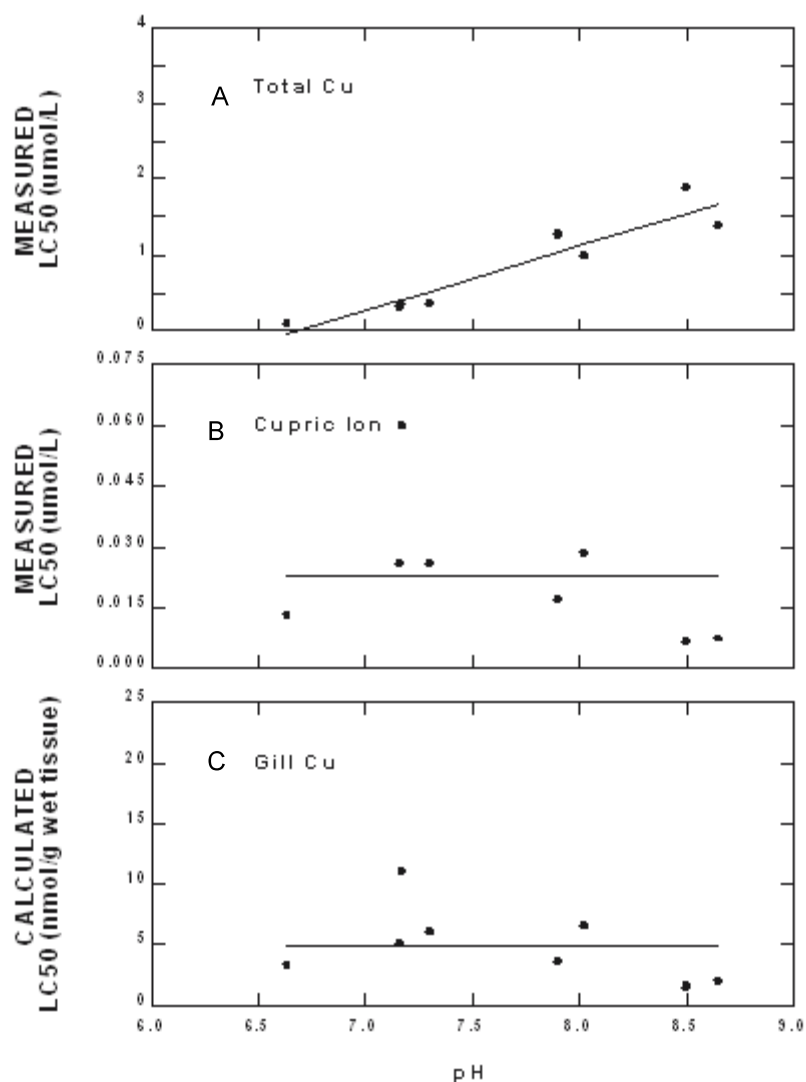


Figure 8 Relationship of copper LC50s to variations in pH. The lines are drawn by eye to represent the data. (A) LC50 expressed as the concentration of total dissolved copper. (B) LC50 expressed as the concentration of the free ion activity of copper. (C) LC50 expressed as the concentration of the copper sorbed to the gill. The LA50s in (B) are the measured copper activities using a specific ion electrode. Data from Erickson et al. (1996).

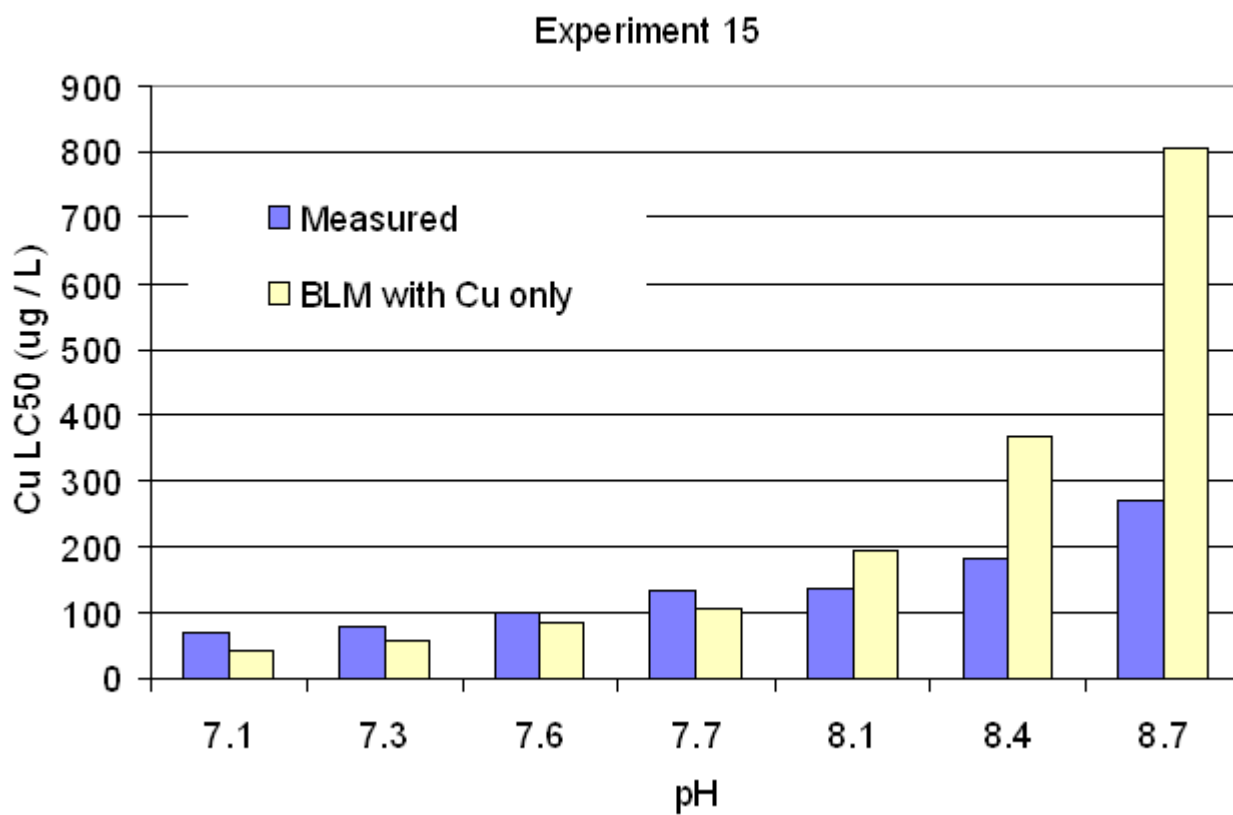


Figure 9 **Response of measured and predicted fathead minnow total Cu LC50 to changes in pH. BLM predictions assume that only Cu²⁺ is bioavailable.**

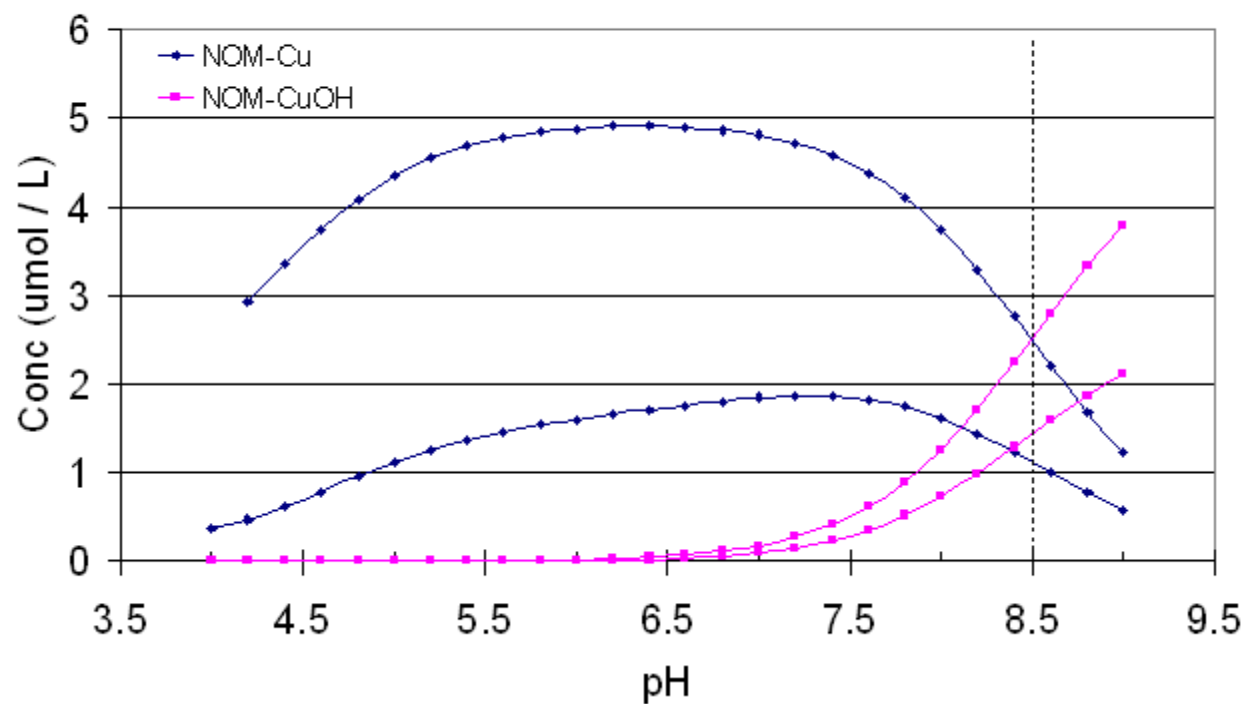


Figure 10 Distribution of complexed Cu^{2+} and CuOH^+ on natural organic matter as simulated in the WHAM.

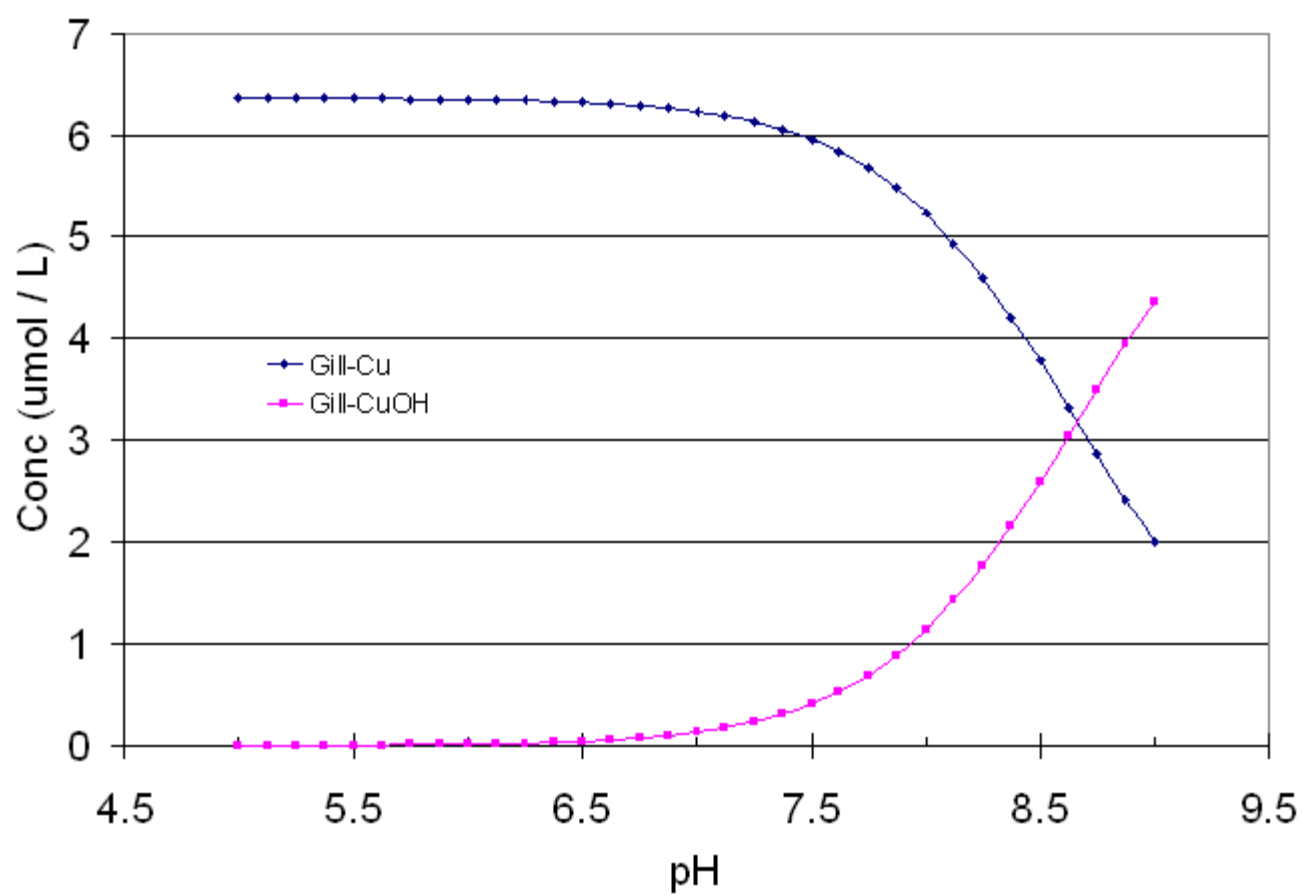


Figure 11 Distribution of adsorbed Cu^{2+} and CuOH^+ on gill membrane as simulated in the revised BLM.

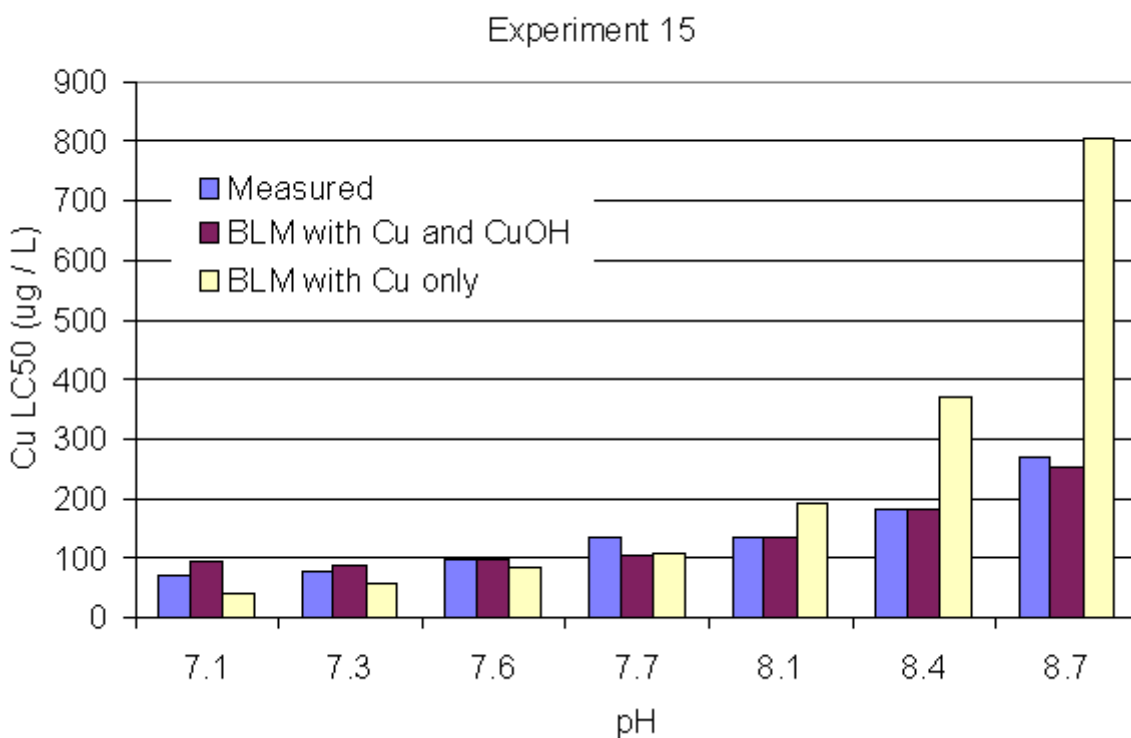


Figure 12 Response of measured and predicted fathead minnow Cu LC50 to changes in pH. BLM predictions assuming only Cu^{2+} is bioavailable are compared with the revised model that assumes both Cu^{2+} and CuOH^+ are bioavailable.

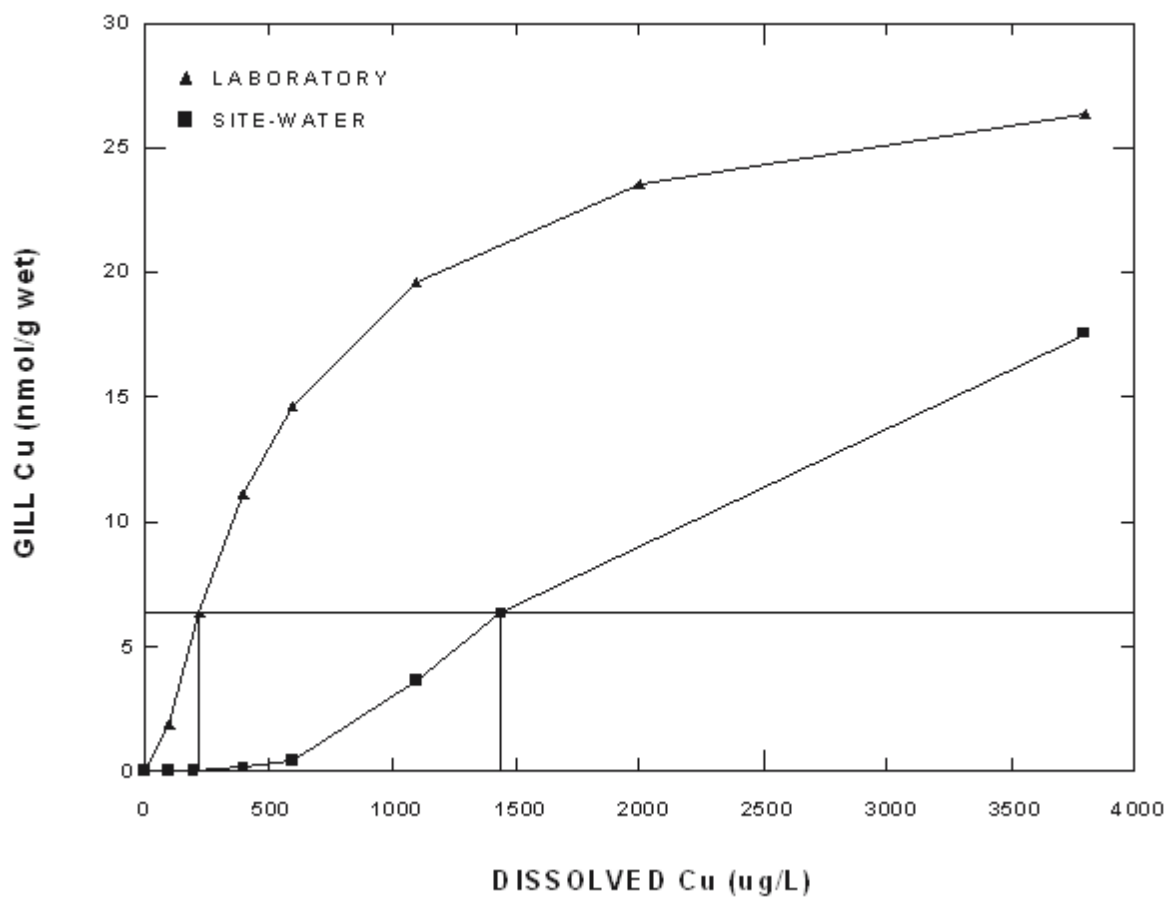


Figure 13 Method of calculating the LC50 using the BLM. Relationship of copper concentration sorbed on the gill and dissolved copper as computed using the BLM for laboratory water and 100% effluent.

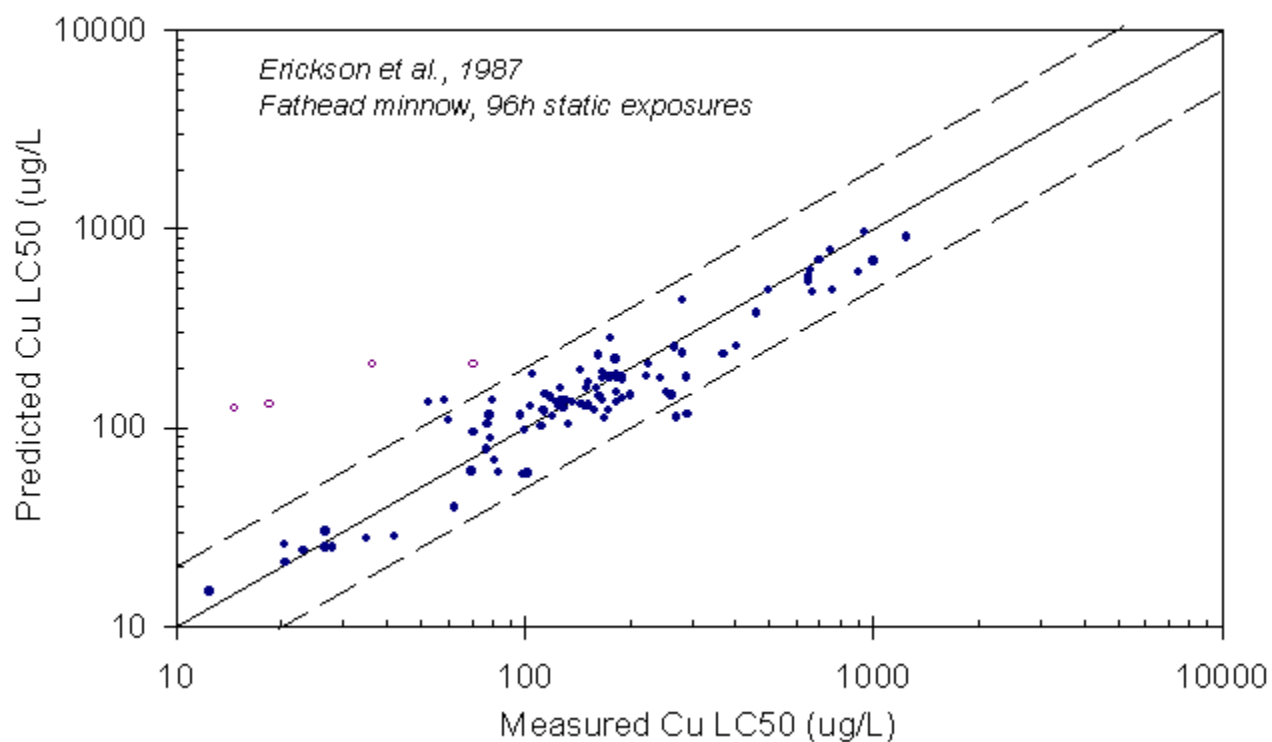


Figure 14 Biotic Ligand Model predicted versus measured LC50 values for fathead minnow in static toxicity exposures from Erickson et al. (1996). The 1:1 (solid), 2:1 and 1:2 (dotted) reference lines are drawn for comparison.

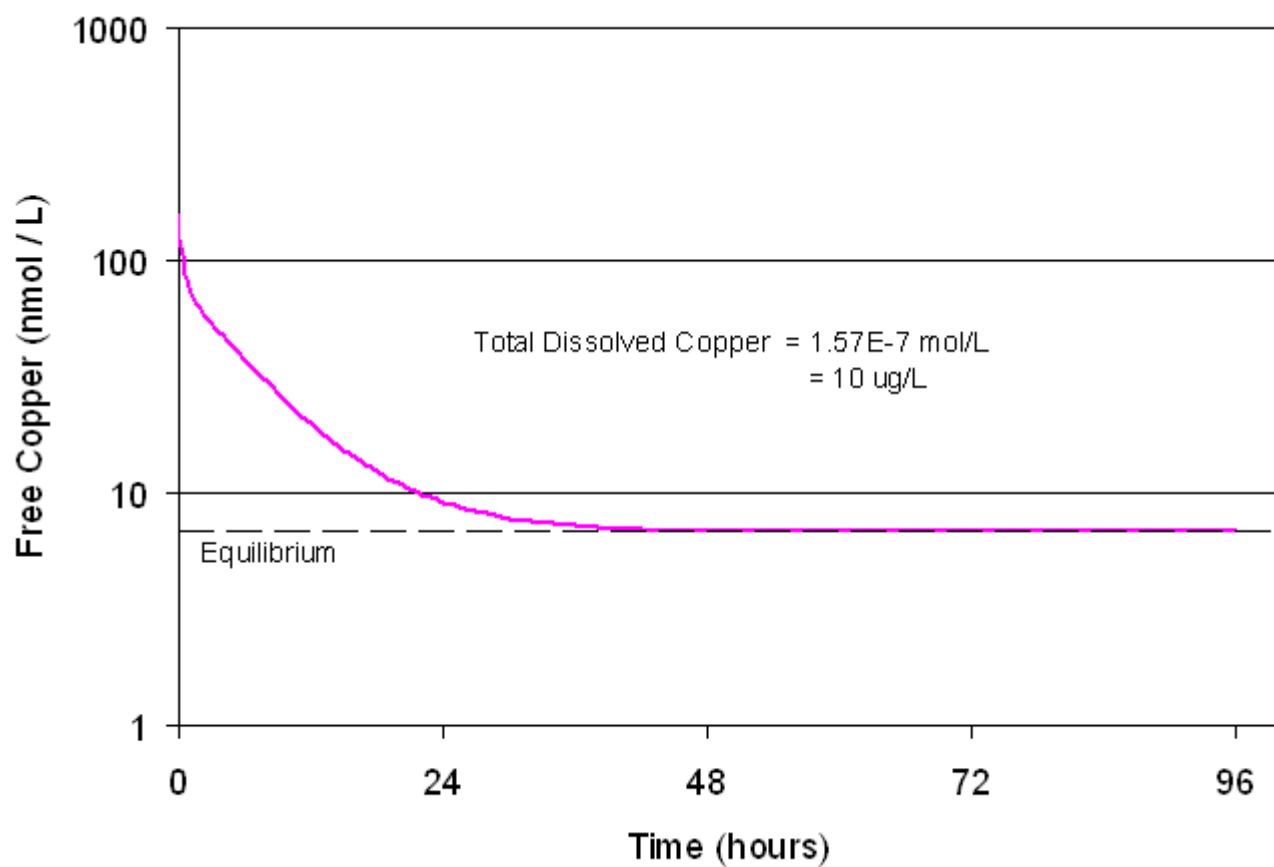


Figure 15 Simulated changes in free copper over time during a static toxicity test. Kinetics of copper binding to natural organic matter are based on results of Ma et al. (1999).

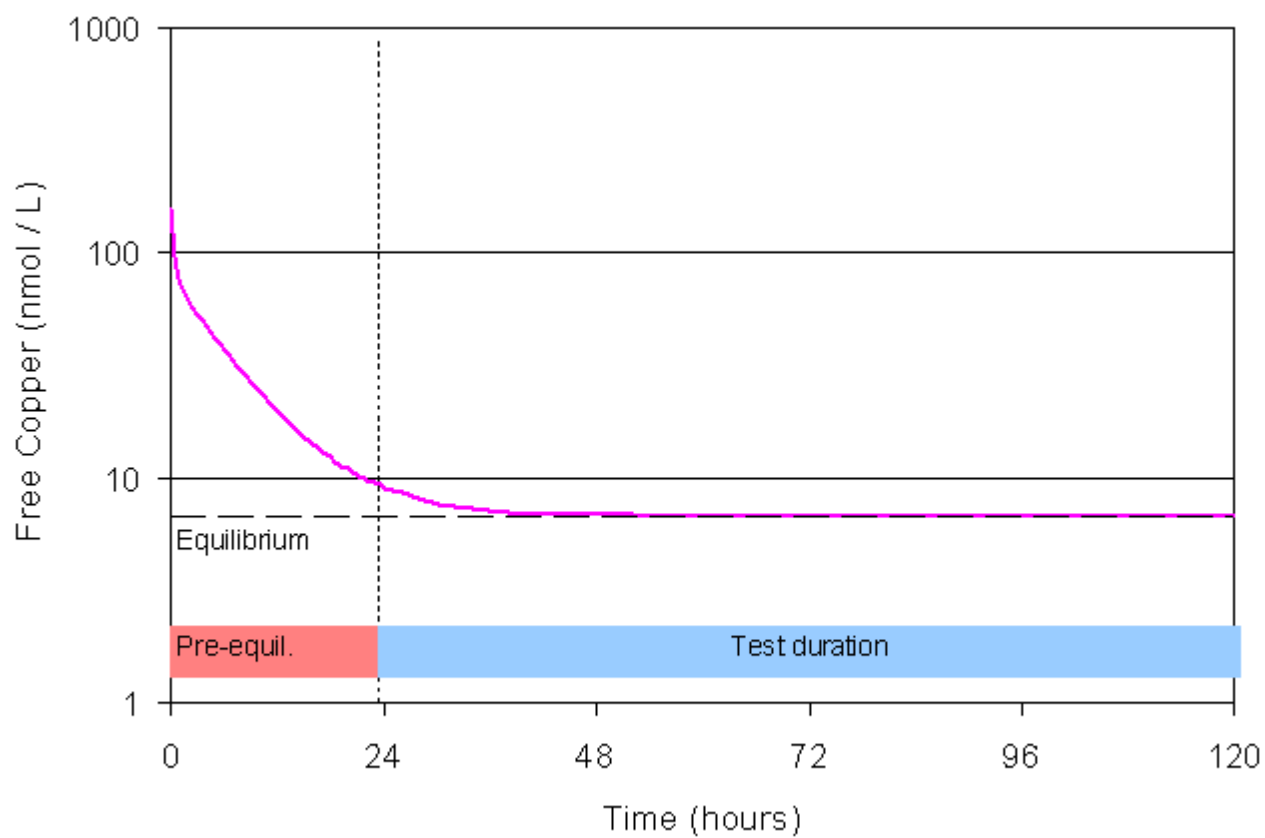


Figure 16 Simulated changes in free copper over time during a static toxicity test with a 24 hour pre-test equilibration period. Kinetics of copper binding to natural organic matter are based on results of Ma et al. (1999).

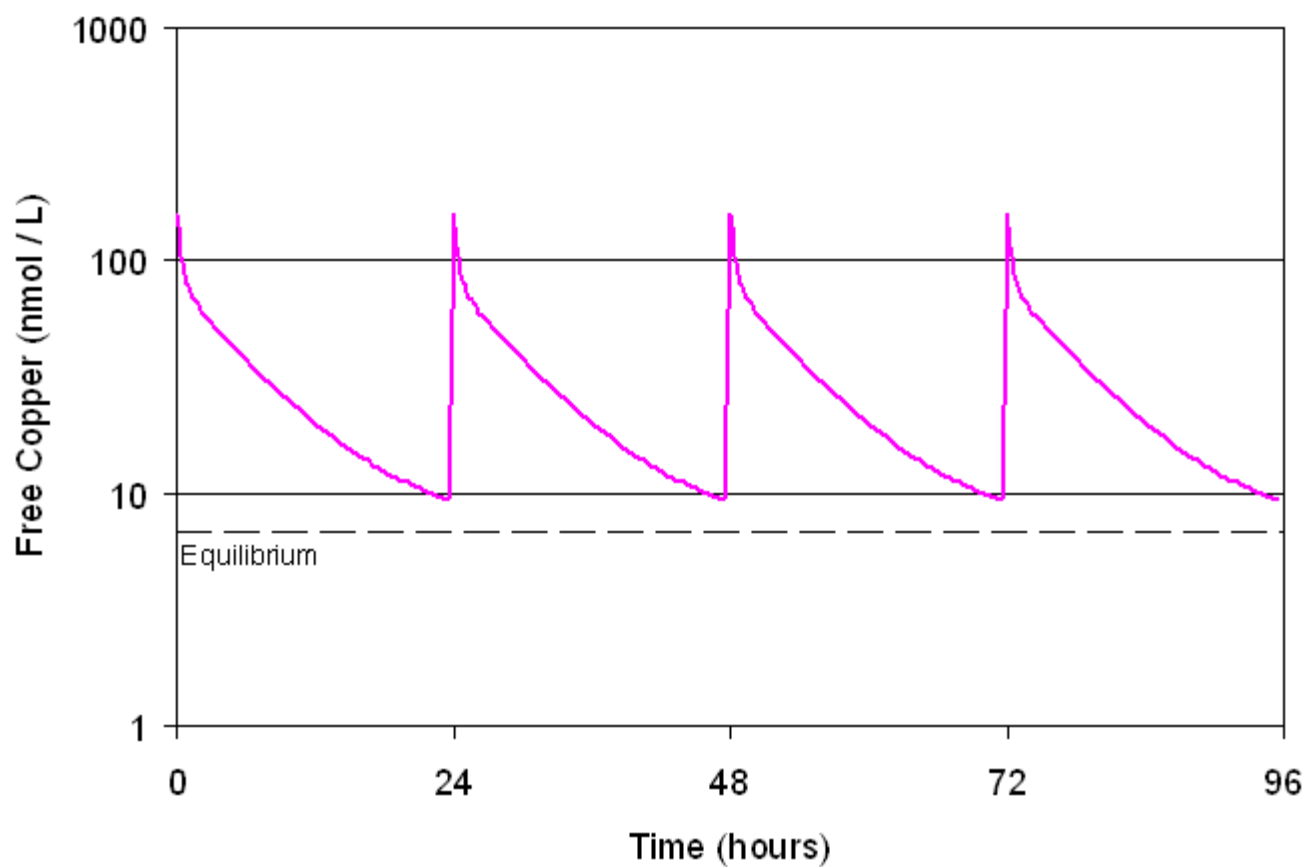


Figure 17 Simulated changes in free copper over time during a static toxicity test with a 24 hour renewal frequency. Kinetics of copper binding to natural organic matter are based on results of Ma et al. (1999).

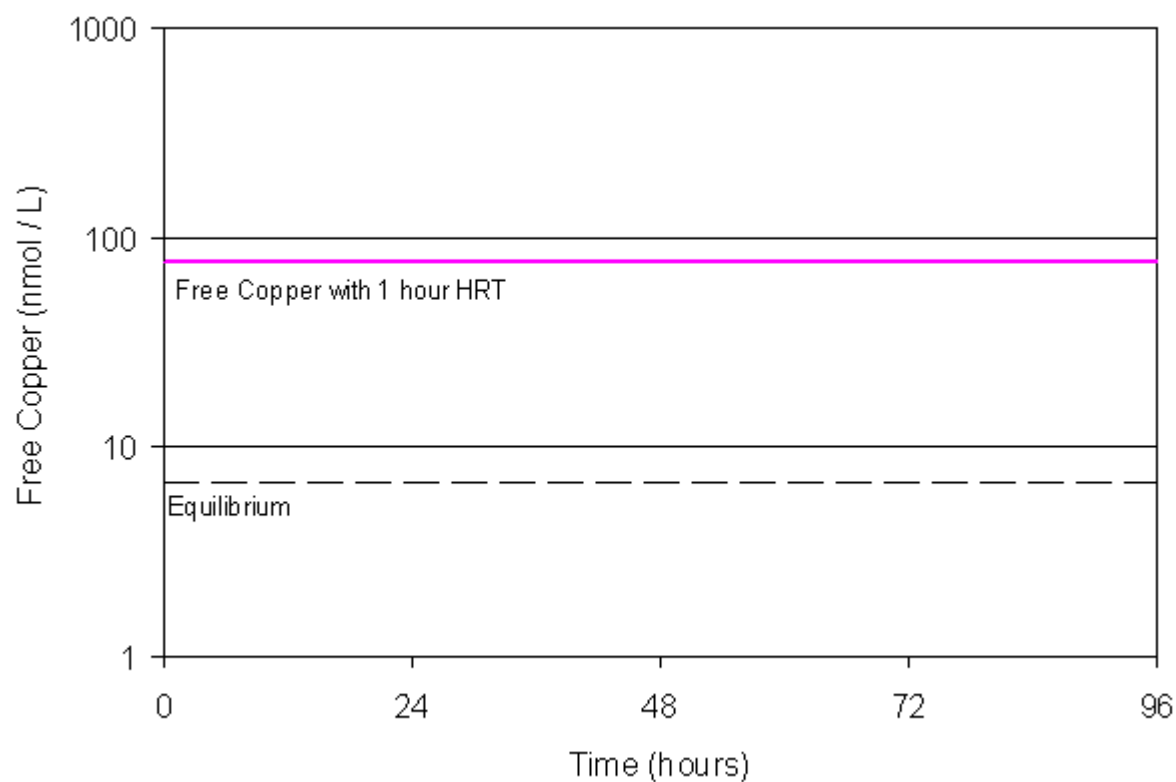


Figure 18 Simulated changes in free copper over time during a flowthrough toxicity test with 1 hour of contact time of the copper in the exposure water. Kinetics of copper binding to natural organic matter are based on results of Ma et al. (1999).

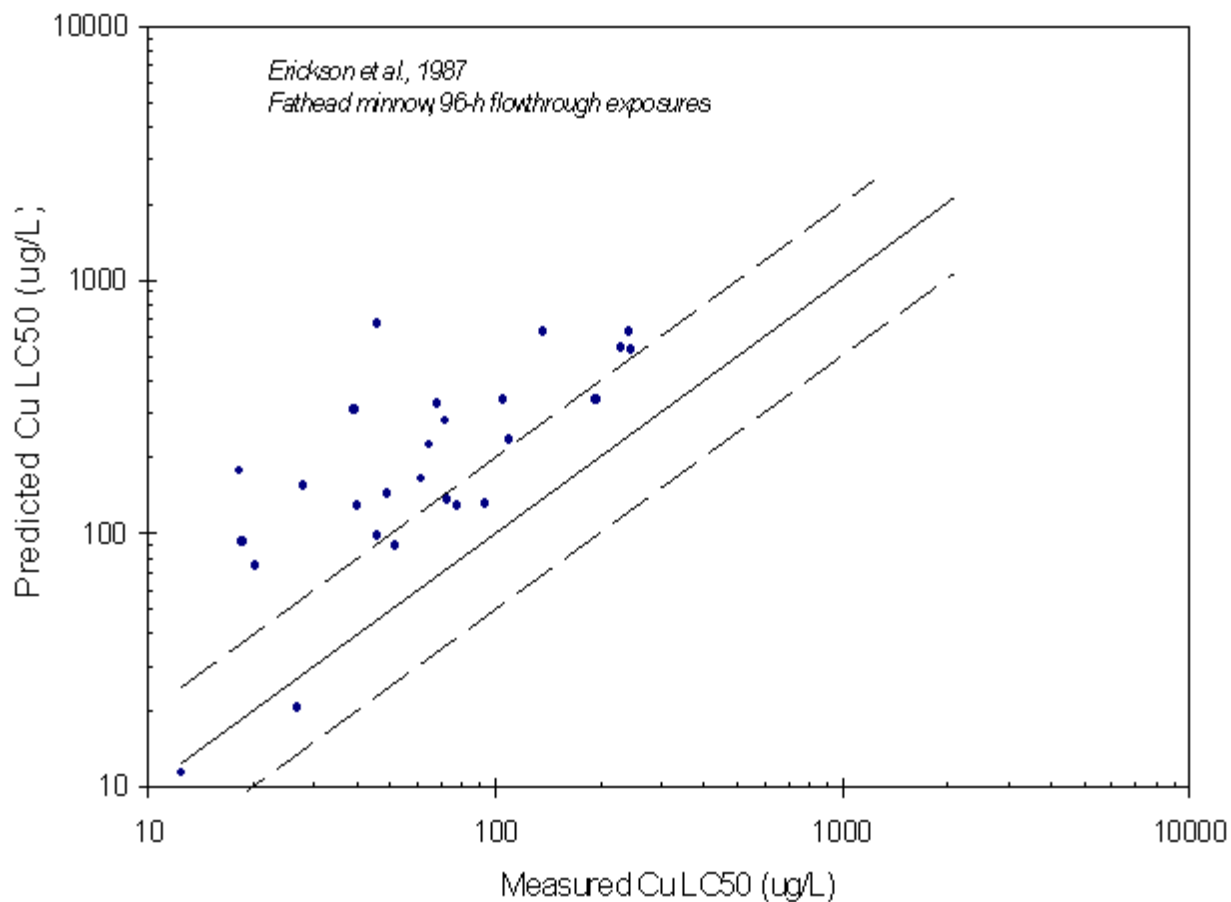


Figure 19 Biotic Ligand Model predicted versus measured LC50 values for fathead minnow in flow-through toxicity exposures from Erickson et al. (1996). The 1:1 (solid), 2:1 and 1:2 (dotted) reference lines are drawn for comparison.

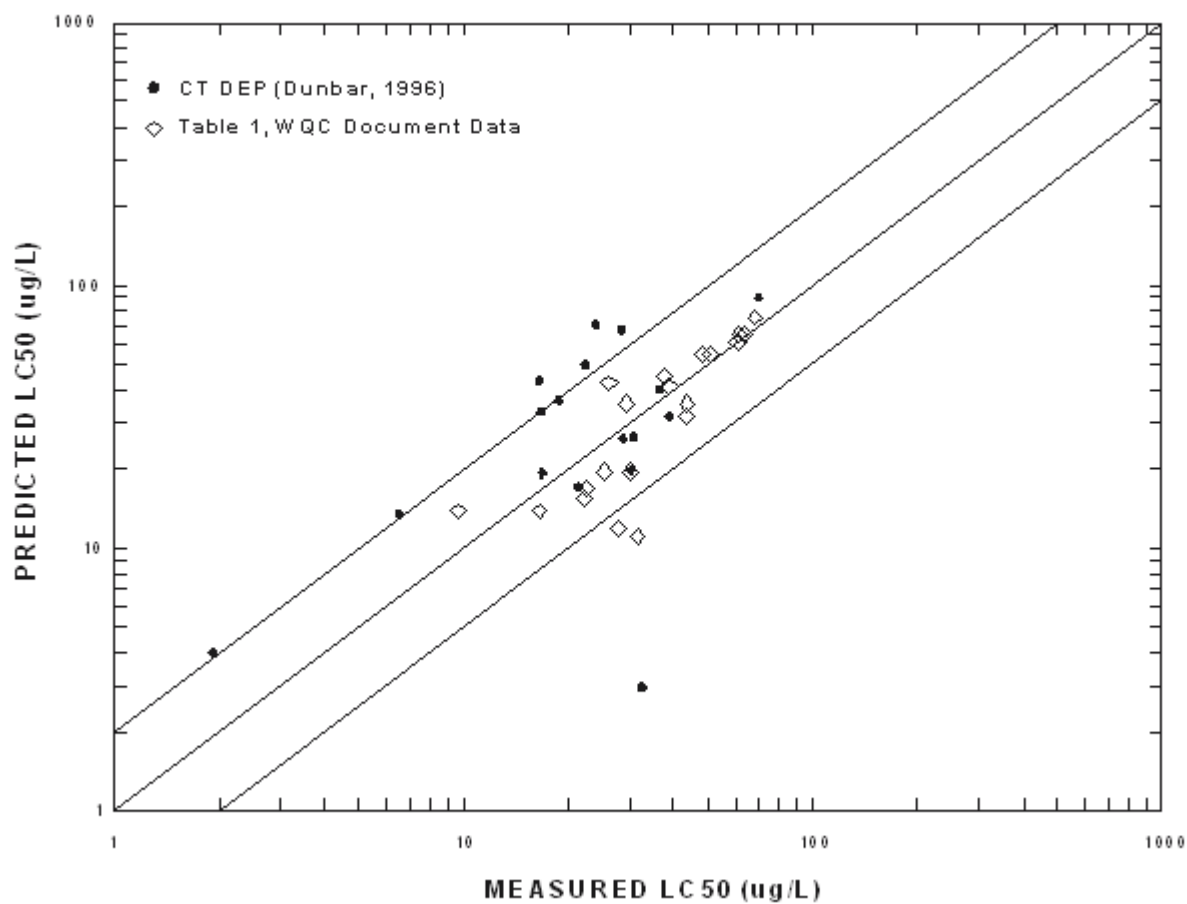


Figure 20 Predicted versus measured values for *D. pulex* copper LC50 in Connecticut streams (CT DEP; 1996b). The 1:1 (solid), 2:1 and 1:2 (dotted) reference lines are drawn for comparison.

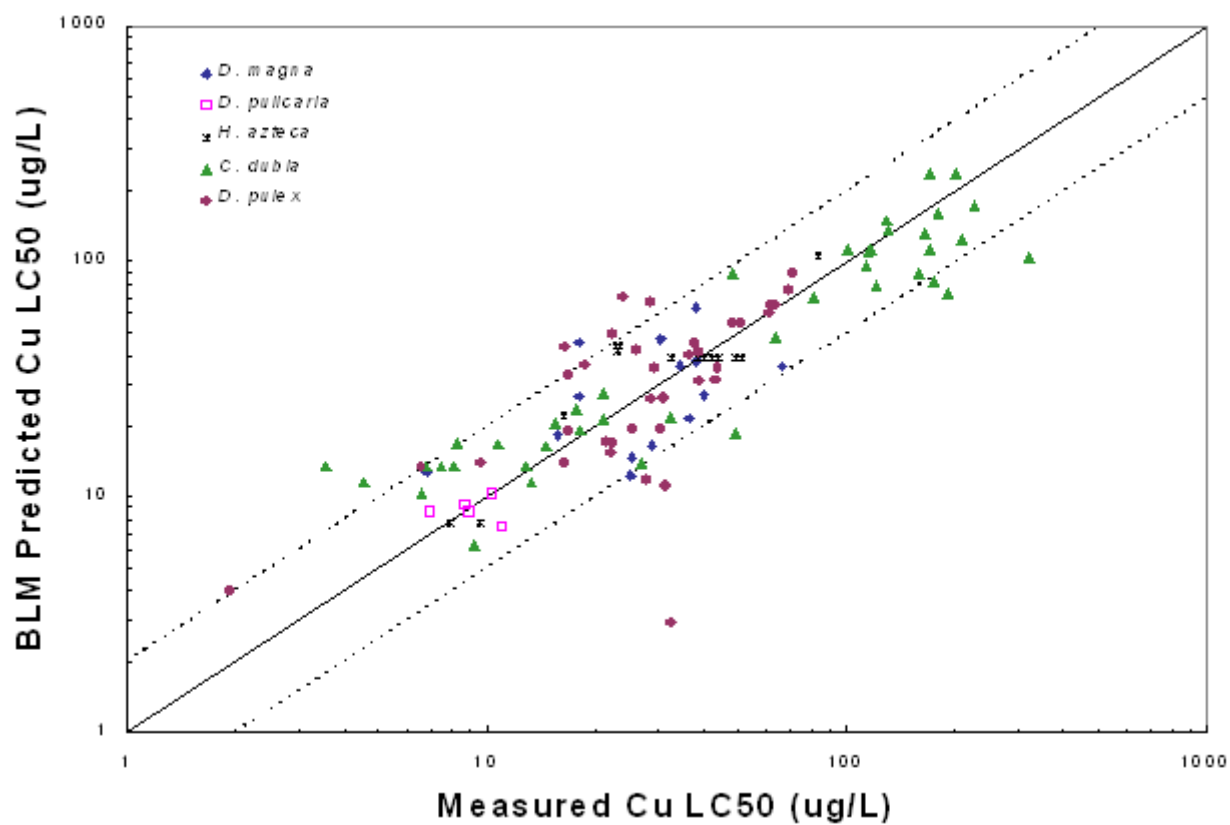
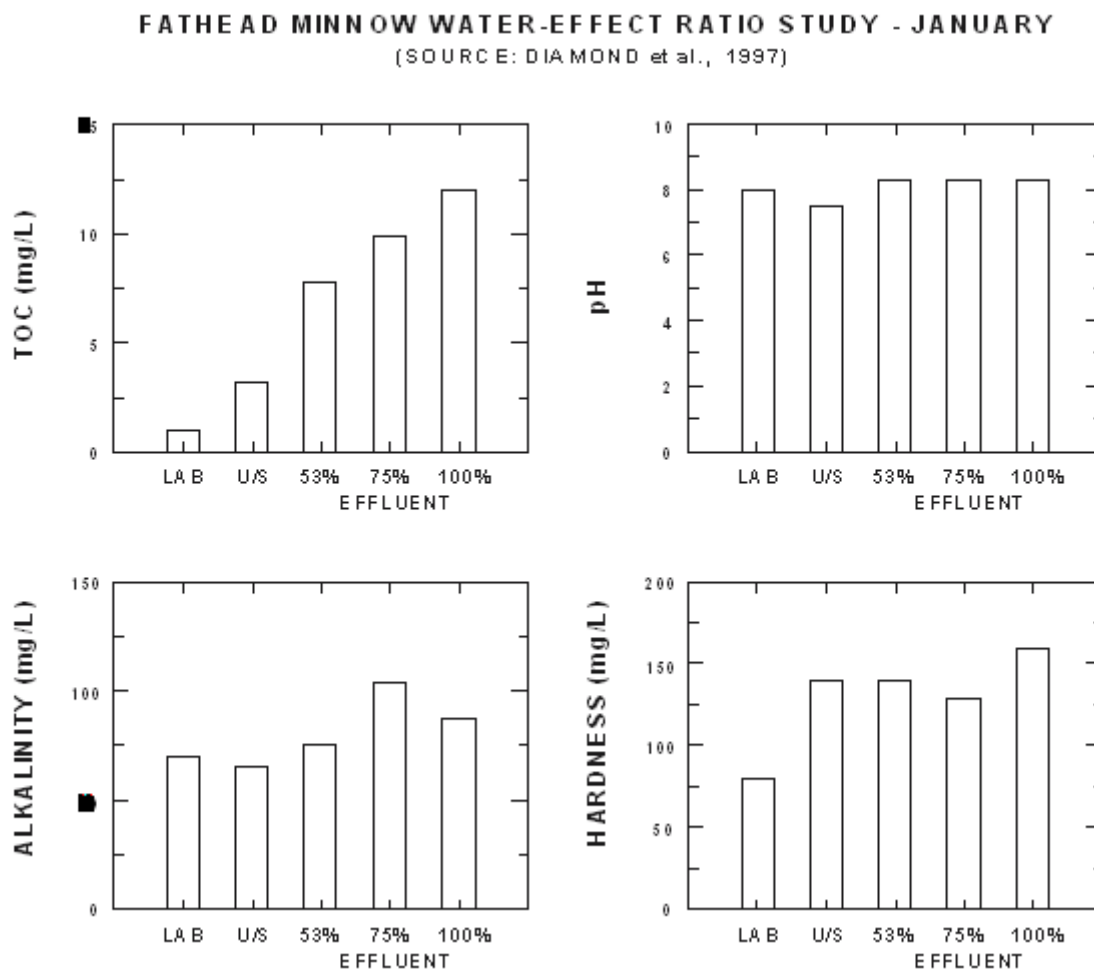


Figure 21 Comparison of BLM predictions for copper toxicity to five aquatic species from the US EPA draft ambient water quality criteria document (USEPA 2003b).

Figure 22 **The aqueous concentrations of TOC (taken to be equivalent to DOC in the application of BLM), alkalinity, hardness, and pH, in laboratory water (LAB), upstream water (U/S), and at the indicated percentages of effluent dilutions in upstream water. Data from Diamond et al. (1997).**



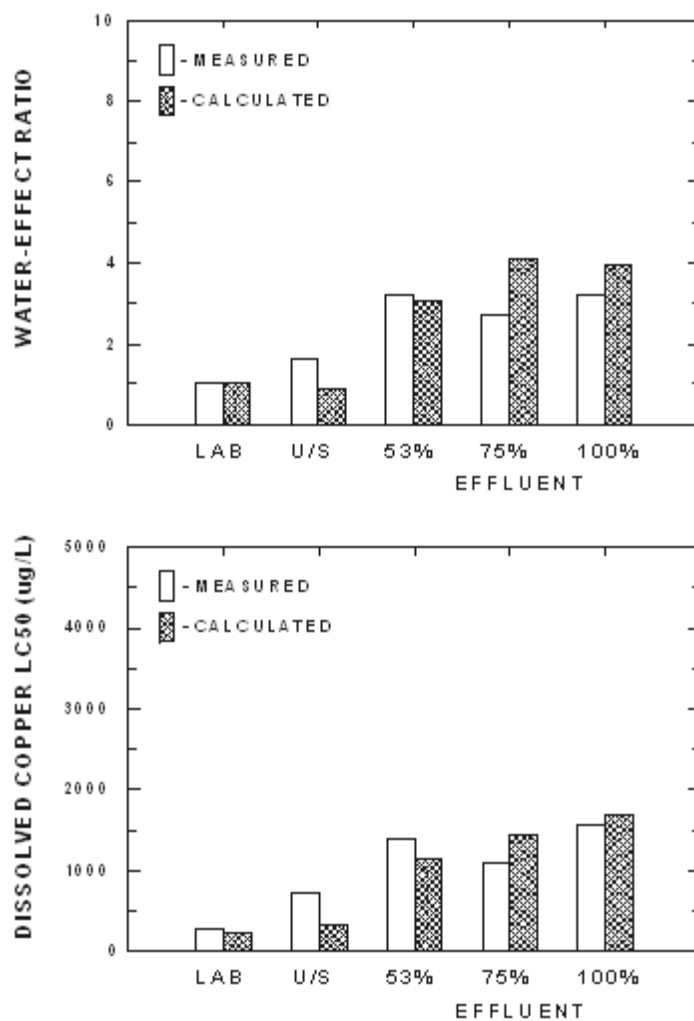


Figure 23 Comparison of measured and calculated LC50s and the water-effect ratios. Data from Diamond et al. (1997).

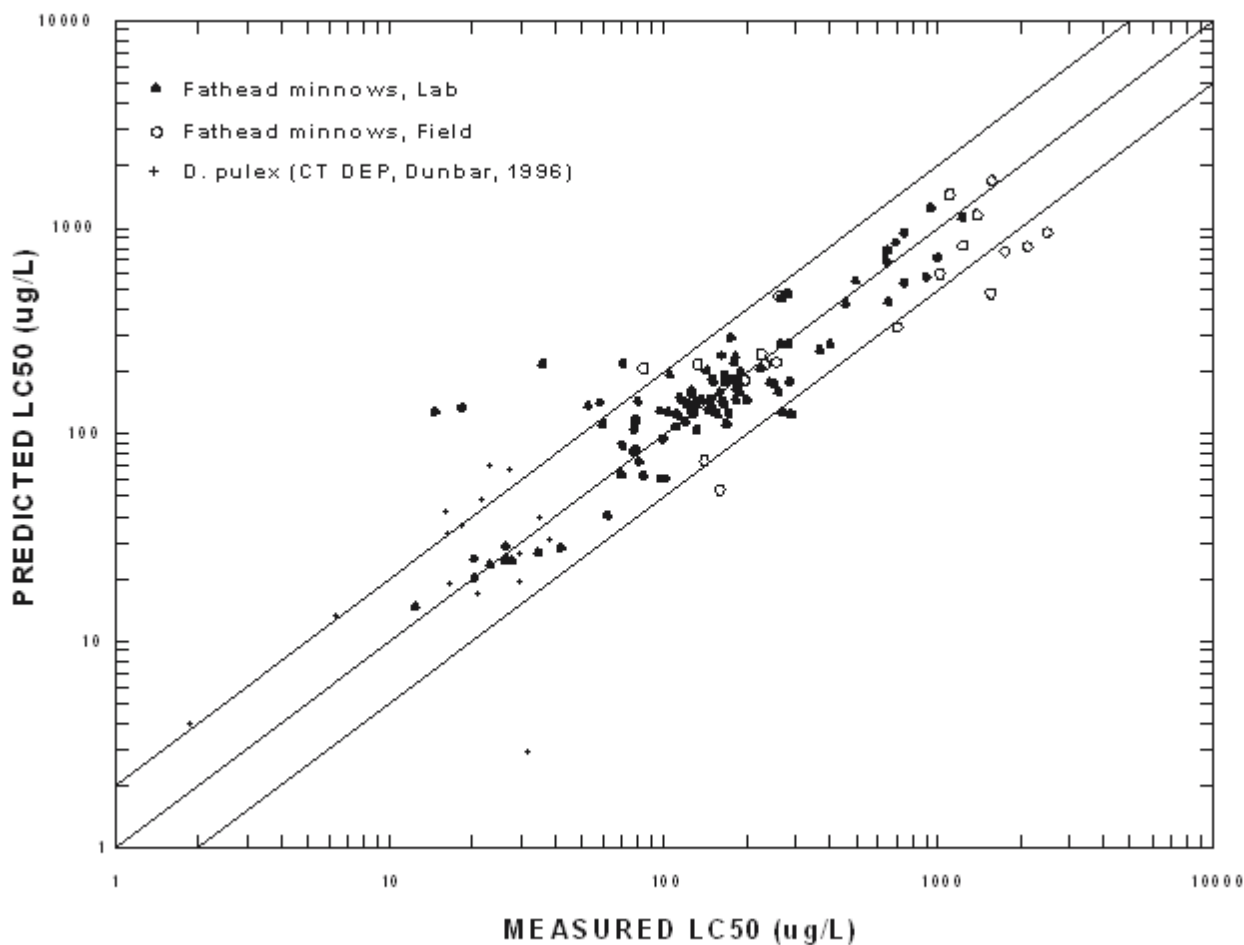


Figure 24 Predicted versus measured values for fathead minnow copper LC50s in water effect ratio studies (Diamond et al., 1997; Dunbar, 1996). The results from static exposures from Erickson et al. (1987; fathead minnow lab) are included for comparison. The 1:1 (solid), 2:1 and 1:2 (dotted) reference lines are drawn for comparison.

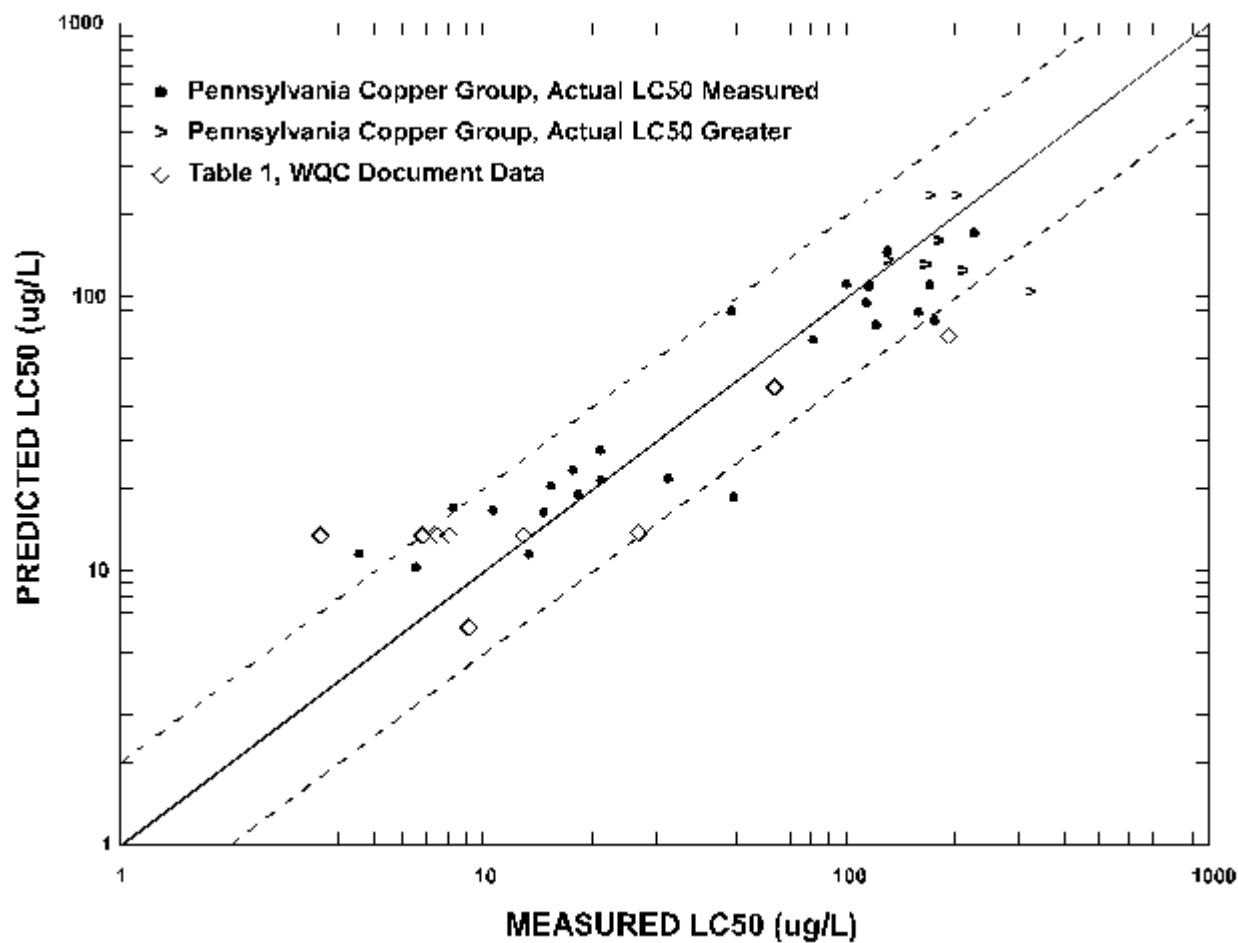


Figure 25 Predicted versus measured values for *C. dubia* copper LC50s in Water Effect Ratio studies (Hall et al., 1998). The 1:1 (solid), 2:1 and 1:2 (dotted) reference lines are drawn for comparison.

APPENDIX A. Chemical Speciation Equations for Copper

APPENDIX A

CHEMICAL SPECIATION EQUATIONS FOR COPPER

The water quality criteria for copper will be developed using the Biotic Ligand Model (BLM; Renner, 1997; Meyer et al., 1999; Di Toro et al., 1999; Paquin et al., 1999; Santore et al., 1999a). The conceptual framework for the BLM is an adaptation of several previous attempts to predict metal toxicity and bioavailability including the Gill Site Interaction Model (GSIM: Pagenkopf, 1983) and recent extensions by Playle and coworkers (Playle et al., 1992, 1993a, b; Janes and Playle, 1995; Wood et al., 1999) and the Free Ion Activity Model (FIAM: Morel, 1983b; Campbell, 1995). The BLM synthesizes these previous efforts and combines these conceptual models with state of the art descriptions of metal chemistry and the latest information on the physiology of metal bioavailability.

The underlying equations in the BLM include descriptions of the amounts of all the chemical components in the model (the mole balance equations), and how these amounts are distributed amongst all of the chemical species possible for each component (the mass action equations). For an examination of the underlying mathematical structure of the model, it is helpful to start with the mole balance relationships. The user of the BLM must specify a total concentration for each chemical component in the model. The total concentrations for these components include T.H, T.Cu, T.DOC, T.Ca, T.Mg, T.Na, T.K, T.SO₄, T.Cl, T.CO₃. For each of the following equations, we will introduce a notation such that the prefix "T" indicates a total concentration, and the suffix identifies the individual chemical components. These total concentrations can also be expressed in matrix notation such that the vector of all total concentrations is expressed as T, and an arbitrary element is identified as T_j. In addition to the components identified as user inputs, the following components, derived from the Windermere Humic Aqueous Model (WHAM: Tipping, 1994), are introduced.

$$T.HA1H = DOM * f * NHA * 0.7 / 5.78$$

$$T.HA2H = DOM * f * NHA * 1.0 / 5.78$$

$$T.HA3H = DOM * f * NHA * 1.0 / 5.78$$

$$T.HA4H = DOM * f * NHA * 0.7 / 5.78$$

$$T.FA1H = DOM * (1 - f) * NFA * 0.7 / 5.78$$

$$T.FA2H = DOM * (1 - f) * NFA * 1.0 / 5.78$$

$$T.FA3H = DOM * (1 - f) * NFA * 1.0 / 5.78$$

$$T.FA4H = DOM * (1 - f) * NFA * 0.7 / 5.78$$

$$T.HB1H = DOM * f * NHA * 0.7 / 5.78$$

$$T.HB2H = DOM * f * NHA * 0.49 / 5.78$$

$$T.HB3H = DOM * f * NHA * 0.49 / 5.78$$

$$T.HB4H = DOM * f * NHA * 0.7 / 5.78$$

$$T.FB1H = DOM * (1 - f) * NFA * 0.7 / 5.78$$

$$T.FB2H = DOM * (1 - f) * NFA * 0.49 / 5.78$$

$$T.FB3H = DOM * (1 - f) * NFA * 0.49 / 5.78$$

$$T. T.FB4H = DOM * (1 - f) * NFA * 0.7 / 5.78$$

Where:

DOM = DOC * 2 / 1000 (units of g/L of Organic Matter)

f = fraction of DOM in humic substances (input by the user)

NHA = Number of reactive sites in humic acids = 3.29×10^{-3} moles per g HA

NFA = Number of reactive sites in fulvic acids = 4.73×10^{-3} moles per g HA

WHAM is a state-of-the-art representation of the interactions between natural organic matter and metals, including copper. The BLM has adopted the chemical description in WHAM for organic matter interactions.

Finally, a component is introduced for mass balance of substances on the gill:

$T_{\text{Gill}} = .000030$ moles per kg wet weight of gill = 30 nmol/g_w

Each of these chemical components can be distributed among a variety of chemical species, such that the sum of all species, with appropriate stoichiometric relations, must equal those total quantities. These mole balance relationships are listed in Table A1.

Together, the mole balance relationships comprise a set of 26 equations, and the proper description of copper chemistry must satisfy all equations simultaneously. A generic expression for these equations can be summarized as follows:

$$T_j = \sum_i S_i a_{i,j}$$

Where the concentration of an individual chemical species is represented by S_i and the stoichiometric coefficient between S_i and T_j is indicated by $a_{i,j}$.

A solution can be obtained by first substituting the species concentrations in each of the mole balance equations. The species concentrations can be expressed as a function of the concentrations of each of the chemical components by the use of mass action expressions. The mass action expressions for the formation of each of the species in the BLM are listed in Table A2. Table A2 contains a description of interactions on a gill suitable for determining copper toxicity to fathead minnow (*Pimephales promelas*). For each reaction, the concentration of a species can be calculated as:

$$S_i = K_i \prod C_k a_{ik}$$

Where the K_i for each species is given in Table A2, the stoichiometric coefficients between species i and component k is $a_{i,k}$, and the concentration of each component C_k is an unknown. The values of K in Table A2 are modified for the specific conditions of ionic strength and temperature. Ionic strength corrections can be provided by the extended Debye-Huckel Equation (Morel, 1983a) for inorganic species, and by a Donnan-layer expression for organic species (Tipping, 1994). Substituting these mass action expressions into the mole balance equations generates a system of 26 equations (T) in 26 unknowns (C). These can be solved simultaneously to derive the final chemical distribution at equilibrium. Conversely, an iterative approach can be applied to solve for the dissolved copper concentration that would be needed for the Cu-biotic ligand concentration to equal the critical effect level that is associated with the LC50. This is the approach that is used in the BLM to predict metal effect levels.

Table A1. Mole Balance Equation for Chemical Components in the BLM

T.H == H + Gill-H

- HA1 - HA2 - HA3 - HA4 - HB1 - HB2 - HB3 - HB4
 - FA1 - FA2 - FA3 - FA4 - FB1 - FB2 - FB3 - FB4

- HA1-Mg - HA2-Mg - HA3-Mg - HA4-Mg - HB1-Mg - HB2-Mg - HB3-Mg - HB4-Mg
 - FA1-Mg - FA2-Mg - FA3-Mg - FA4-Mg - FB1-Mg - FB2-Mg - FB3-Mg - FB4-Mg

- HA1-Ca - HA2-Ca - HA3-Ca - HA4-Ca - HB1-Ca - HB2-Ca - HB3-Ca - HB4-Ca
 - FA1-Ca - FA2-Ca - FA3-Ca - FA4-Ca - FB1-Ca - FB2-Ca - FB3-Ca - FB4-Ca

- HA1-Cu - HA2-Cu - HA3-Cu - HA4-Cu - HB1-Cu - HB2-Cu - HB3-Cu - HB4-Cu
 - FA1-Cu - FA2-Cu - FA3-Cu - FA4-Cu - FB1-Cu - FB2-Cu - FB3-Cu - FB4-Cu

+ HCO₃ + 2H₂CO₃ + MgHCO₃ + CaHCO₃ + CuHCO₃

- HA1-CuOH - HA2-CuOH - HA3-CuOH - HA4-CuOH
 - HB1-CuOH - HB2-CuOH - HB3-CuOH - HB4-CuOH
 - FA1-CuOH - FA2-CuOH - FA3-CuOH - FA4-CuOH
 - FB1-CuOH - FB2-CuOH - FB3-CuOH - FB4-CuOH

T.Cu = + Cu + Gill-Cu

+ HA1-Cu + HA2-Cu + HA3-Cu + HA4-Cu + HB1-Cu + HB2-Cu + HB3-Cu + HB4-Cu
 + FA1-Cu + FA2-Cu + FA3-Cu + FA4-Cu + FB1-Cu + FB2-Cu + FB3-Cu + FB4-Cu

+ CuOH + Cu(OH)₂
 + HA1-CuOH + HA2-CuOH + HA3-CuOH + HA4-CuOH
 + HB1-CuOH + HB2-CuOH + HB3-CuOH + HB4-CuOH
 + FA1-CuOH + FA2-CuOH + FA3-CuOH + FA4-CuOH
 + FB1-CuOH + FB2-CuOH + FB3-CuOH + FB4-CuOH

+ CuSO₄ + CuCO₃ + Cu(CO₃)₂ + CuCl + CuHCO₃

T.Ca == Ca + Gill-Ca

+ HA1-Ca + HA2-Ca + HA3-Ca + HA4-Ca + HB1-Ca + HB2-Ca + HB3-Ca + HB4-Ca
 + FA1-Ca + FA2-Ca + FA3-Ca + FA4-Ca + FB1-Ca + FB2-Ca + FB3-Ca + FB4-Ca

+ CaHCO₃ + CaCO₃ + CaSO₄

Table A1. Continued

T.Mg = + Mg

+ HA1-Mg + HA2-Mg + HA3-Mg + HA4-Mg + HB1-Mg + HB2-Mg + HB3-Mg
+ HB4-Mg + FA1-Mg + FA2-Mg + FA3-Mg + FA4-Mg + FB1-Mg + FB2-Mg
+ FB3-Mg + FB4-Mg

+ MgHCO₃ + MgCO₃ + MgSO₄

T.Na = + Na + Gill-Na

T.K = + K

T.SO₄ = + SO₄ + MgSO₄ + CaSO₄ + CuSO₄

T.Cl = + Cl + CuCl

T.CO₃ = + CO₃ + HCO₃ + H₂CO₃ + MgHCO₃ + MgCO₃
+ CaHCO₃ + CaCO₃ + CuCO₃ + 2Cu(CO₃)₂ + CuHCO₃

T.HA1H = + HA1H + HA1 + HA1-Mg + HA1-Ca + HA1-Cu + HA1-CuOH

T.HA2H = + HA2H + HA2 + HA2-Mg + HA2-Ca + HA2-Cu + HA2-CuOH

T.HA3H = + HA3H + HA3 + HA3-Mg + HA3-Ca + HA3-Cu + HA3-CuOH

T.HA4H = + HA4H + HA4 + HA4-Mg + HA4-Ca + HA4-Cu + HA4-CuOH

T.HB1H = + HB1H + HB1 + HB1-Mg + HB1-Ca + HB1-Cu + HB1-CuOH

T.HB2H = + HB2H + HB2 + HB2-Mg + HB2-Ca + HB2-Cu + HB2-CuOH

T.HB3H = + HB3H + HB3 + HB3-Mg + HB3-Ca + HB3-Cu + HB3-CuOH

T.HB4H = + HB4H + HB4 + HB4-Mg + HB4-Ca + HB4-Cu + HB4-CuOH

T.FA1H = + FA1H + FA1 + FA1-Mg + FA1-Ca + FA1-Cu + FA1-CuOH

T.FA2H = + FA2H + FA2 + FA2-Mg + FA2-Ca + FA2-Cu + FA2-CuOH

T.FA3H = + FA3H + FA3 + FA3-Mg + FA3-Ca + FA3-Cu + FA3-CuOH

T.FA4H = + FA4H + FA4 + FA4-Mg + FA4-Ca + FA4-Cu + FA4-CuOH

T.FB1H = + FB1H + FB1 + FB1-Mg + FB1-Ca + FB1-Cu + FB1-CuOH

T.FB2H = + FB2H + FB2 + FB2-Mg + FB2-Ca + FB2-Cu + FB2-CuOH

T.FB3H = + FB3H + FB3 + FB3-Mg + FB3-Ca + FB3-Cu + FB3-CuOH

T.FB4H = + FB4H + FB4 + FB4-Mg + FB4-Ca + FB4-Cu + FB4-CuOH

T.Gill = + Gill + Gill-Cu + Gill-Ca + Gill-H + Gill-Na

Table A2. Species Formation Reactions

Species Formation Reaction	Log K	Species Formation Reaction	Log K
Gill-Cu = + Gill + Cu	7.4	HA1-Ca = + HA1H - H + Ca	-3.2
Gill-Ca = + Gill + Ca	3.6	HA2-Ca = + HA2H - H + Ca	-3.2
Gill-H = + Gill + H	5.4	HA3-Ca = + HA3H - H + Ca	-3.2
Gill-Na = + Gill + Na	3.0	HA4-Ca = + HA4H - H + Ca	-3.2
HA1 = + HA1H - H	-4.91	HB1-Ca = + HB1H - H + Ca	-3.2
HA2 = + HA2H - H	-4.316667	HB2-Ca = + HB2H - H + Ca	-3.2
HA3 = + HA3H - H	-3.723333	HB3-Ca = + HB3H - H + Ca	-3.2
HA4 = + HA4H - H	-3.13	HB4-Ca = + HB4H - H + Ca	-3.2
HB1 = + HB1H - H	-10.265	A1-Ca = + FA1H - H + Ca	-2.2
HB2 = + HB2H - H	-9.121667F	FA2-Ca = + FA2H - H + Ca	-2.2
HB3 = + HB3H - H	-7.978333	FA3-Ca = + FA3H - H + Ca	-2.2
HB4 = + HB4H - H	-6.835	FA4-Ca = + FA4H - H + Ca	-2.2
FA1 = + FA1H - H	-4.93	FB1-Ca = + FB1H - H + Ca	-2.2
FA2 = + FA2H - H	-3.816667	FB2-Ca = + FB2H - H + Ca	-2.2
FA3 = + FA3H - H	-2.703333	FB3-Ca = + FB3H - H + Ca	-2.2
FA4 = + FA4H - H	-1.59	FB4-Ca = + FB4H - H + Ca	-2.2
FB1 = + FB1H - H	-12.4	HA1-Cu = + HA1H - H + Cu	-1.5
FB2 = + FB2H - H	-10.56	HA2-Cu = + HA2H - H + Cu	-1.5
FB3 = + FB3H - H	-8.72	HA3-Cu = + HA3H - H + Cu	-1.5
FB4 = + FB4H - H	-6.88	HA4-Cu = + HA4H - H + Cu	-1.5
HA1-Mg = + HA1H - H + Mg	-3.3	HB1-Cu = + HB1H - H + Cu	-1.5
HA2-Mg = + HA2H - H + Mg	-3.3	HB2-Cu = + HB2H - H + Cu	-1.5
HA3-Mg = + HA3H - H + Mg	-3.3	HB3-Cu = + HB3H - H + Cu	-1.5
HA4-Mg = + HA4H - H + Mg	-3.3	HB4-Cu = + HB4H - H + Cu	-1.5
HB1-Mg = + HB1H - H + Mg	-3.3	A1-Cu = + FA1H - H + Cu	-0.8
HB2-Mg = + HB2H - H + Mg	-3.3	A2-Cu = + FA2H - H + Cu	-0.8
HB3-Mg = + HB3H - H + Mg	-3.3		
HB4-Mg = + HB4H - H + Mg	-3.3		
FA1-Mg = + FA1H - H + Mg	-2.2	FA3-Cu = + FA3H - H + Cu	-0.8
FA2-Mg = + FA2H - H + Mg	-2.2	FA4-Cu = + FA4H - H + Cu	-0.8
FA3-Mg = + FA3H - H + Mg	-2.2	FB1-Cu = + FB1H - H + Cu	-0.8
FA4-Mg = + FA4H - H + Mg	-2.2	FB2-Cu = + FB2H - H + Cu	-0.8
FB1-Mg = + FB1H - H + Mg-2.2		FB3-Cu = + FB3H - H + Cu	-0.8
FB2-Mg = + FB2H - H + Mg	-2.2	FB4-Cu = + FB4H - H + Cu	-0.8
FB3-Mg = + FB3H - H + Mg-2.2			
FB4-Mg = + FB4H - H + Mg-2.2			

Table A2. (Continued)

Species Formation Reaction	Log K	Species Formation Reaction	Log K
HCO ₃ = + H + CO ₃	10.329	FA1-CuOH = + FA1H - H + Cu	-.8
H ₂ CO ₃ = + 2H + CO ₃	16.681	FA2-CuOH = + FA2H - H + Cu	-.8
MgHCO ₃ = + H + Mg + CO ₃	11.4	FA3-CuOH = + FA3H - H + Cu	-.8
MgCO ₃ = + Mg + CO ₃	2.98	FA4-CuOH = + FA4H - H + Cu	-.8
MgSO ₄ = + Mg + SO ₄	2.37	FB1-CuOH = + FB1H - H + Cu	-.8
		FB2-CuOH = + FB2H - H + Cu	-.8
CaHCO ₃ = + H + Ca + CO ₃	11.44	FB3-CuOH = + FB3H - H + Cu	-.8
CaCO ₃ = + Ca + CO ₃	3.22	FB4-CuOH = + FB4H - H + Cu	-.8
CaSO ₄ = + Ca + SO ₄	2.3		
CuOH = + Cu	6.48	Cu(OH) ₂ = + Cu	11.78
HA1-CuOH = + HA1H - H + Cu	-1.5	CuSO ₄ = + Cu + SO ₄	2.36
HA2-CuOH = + HA2H - H + Cu	-1.5	CuCO ₃ = + Cu + CO ₃	6.75
HA3-CuOH = + HA3H - H + Cu	-1.5	Cu(CO ₃) ₂ = + Cu + 2CO ₃	9.92
HA4-CuOH = + HA4H - H + Cu	-1.5	CuCl = + Cu + Cl	.4
HB1-CuOH = + HB1H - H + Cu	-1.5	CuHCO ₃ = + H + Cu + CO ₃	14.62
HB2-CuOH = + HB2H - H + Cu	-1.5		
HB3-CuOH = + HB3H - H + Cu	-1.5		
HB4-CuOH = + HB4H - H + Cu	-1.5		

The predicted LC50 values can be used to modify a water quality criterion for site-specific conditions (Santore et al., 1999b). The USEPA allows the use of a water effect ratio procedure using LC50 values measured in a site water. The BLM predicted LC50 values can be used in the same way. One possible method of criteria development, is to use the LC50 predicted for fathead minnow compared to the species mean acute value (SMAV) obtained for this organism in laboratory waters (111 ug Cu/L). The WER would then be determined as :

$$\text{WER} = \text{LC50}_{\text{BLM}} / \text{SMAV}$$

The WER would then be used to modify the water quality criteria for copper based on site-specific conditions. The WQC in this case would be the secondary water criteria for copper listed in Table A3.

$$\text{WQCSITE} = \text{WQC} * \text{WER}$$

Application of the BLM to a variety of water quality conditions has been performed and is included here for reference (Table A3). A range of values for chemical concentrations in typical freshwaters was input to the BLM and the copper toxicity and WER values were determined.

**Table A3. Predicted WERs for Representative Ranges in the
Chemistry of Typical Freshwater Bodies**

Water Chemistry					Effect on Toxicity	
DOC mg/L	pH	Ca mg/L	Na mg/L	Alk mg/L	Cu LC50 ug/L	Cu WER
1.0	6.0	12.0	4.6	40	80.6	0.7
1.0	6.0	12.0	4.6	80	123.8	1.1